



**ECOLOGICAL ENERGETICS AND REPRODUCTION IN THE
COMMON RINGTAIL POSSUM, *Pseudocheirus peregrinus*
(MARSUPIALIA: PHALANGEROIDEA)**

by

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge and belief, contains no copy or paraphrase of material previously published or written by another person, except when due reference is made in the text of the thesis.

A handwritten signature in black ink that reads "Sarah Munks". The signature is written in a cursive style, with the first letter of "Sarah" being a large, stylized 'S'.

Sarah Munks

ABSTRACT

This study examines the annual cycle of energy and time expenditure in a small folivorous marsupial, the common ringtail possum, *Pseudocheirus peregrinus*. Particular attention was given to the energy expended in lactation by the females. Field metabolic rate (FMR) and water flux were measured using an isotopic technique (doubly labeled water). Feeding rates were estimated from measurements of FMR in conjunction with information on the composition of the diet and a digestibility study. FMR's and subsequent feeding rates estimated by the doubly-labeled water technique do not include the proportion of food consumed which is diverted to milk solids and is not metabolised by the mother. The amount of energy transferred directly to the young ringtail possum via the milk was estimated from measurements of milk composition and production.

Reproduction in the common ringtail possum in Tasmania was seasonal, with the majority of births (mean litter size 1.8) in late autumn and early winter. In general, the young leave the pouch during early spring and are fully weaned by the early summer months.

There was no significant seasonal variation in the energy expenditure or water influx of males. The mean FMR of males and non-lactating females was approximately 2.5 times basal metabolic rate which is consistent with the hypothesis that a low total energy cost of free existence (or field metabolic rate) is a characteristic shared by arboreal folivores. Females showed significant changes in water influx and energy expenditure according to their lactational status. The greatest metabolisable energy expenditure was that of females during Phase 3 of lactation (30% above non-reproductive metabolism). Water influx was correspondingly high in these females (36% above non-lactating females).

In general, ringtail possums in both the field and captivity lactated for approximately seven months. However, the length of lactation was shorter in females which bred twice in a year. The composition of the milk varied throughout lactation. A peak in milk solids and energy content coincided with emergence of the young from the pouch. Milk solids represented around 18% (w/w) with milk fat representing only 10% of milk solids. Milk production peaked during Phase 3 of lactation. The dilute milk with a relatively low fat content combined with a long period of lactation result in slow growth of the young.

Peak milk energy output was $154.5 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$ and peak metabolisable energy allocation during lactation was $763.2 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$. These were lower than values available for other herbivores. However, the total output of milk energy by ringtail possums (11 MJ/kg) and total metabolisable energy allocation during reproduction (23.4 MJ/kg) were similar to estimates available for other herbivores. The lactational strategy of the ringtail possum has been selected, most likely, in order to spread the energy demands of reproduction over time due to constraints on the rate of energy intake imposed by a leaf diet. The total energy requirement for reproduction

(34.4 MJ/year, or 14% of total annual energy budget) suggests that the ringtail also has a relatively low overall energy investment in reproduction.

Estimates of total body water made from isotopic dilution and measurements of body mass suggest that females utilise body fat stored during the early stages of lactation to cope with the additional energy required for late lactation. However, reproduction is apparently timed such that late lactation coincides with the increased production of young foliage. Therefore females may also increase their food and water intake during late lactation by consuming young foliage.

Differences were found in the composition of milk collected from wild and captive animals. Chemical analyses of the leaves eaten suggested that these differences were due to variations in diet composition. It was, therefore, proposed that the intra- and inter-population variation in reproductive traits shown by the common ringtail possum may be related to variations in milk composition and/or production caused by variations in diet quality.

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CHAPTER 1

GENERAL INTRODUCTION

Despite the apparent abundance of foliage as a potential food source, relatively few vertebrates use leaves as dietary items. The reason for this appears to be related to the chemical composition of foliage (McNab, 1978; Hume, 1982); in particular, its high fibre (cell-wall) content, low protein content and the presence of 'allelochemicals' (McNab, 1978; Oates *et al.*, 1980; Cork, 1984; Cork and Pahl, 1984). The latter are non-nutritional compounds such as essential oils and phenols (Feeny, 1970; Cork, 1984) produced by trees, which adversely affect other organisms and hence protect leaves to some extent from being eaten (Freeland and Janzen, 1974; Reese, 1979; Coley *et al.*, 1985). These characteristics combine to lower the nutritional value of tree foliage relative to other types of food.

In theory, energetic and morphological constraints need to be overcome by arboreal mammals if they are to utilise tree foliage efficiently as a food source (Cork and Sanson, 1990). The potential for rapid extraction of energy from leaves by a mammalian folivore is low, due to the high fibre content (Van Soest, 1982; Cork, 1984; Hume *et al.*, 1984; Cork and Sanson, 1990). In addition, the proportion of the gross energy content of leaves made up by allelochemicals may not be metabolisable by a particular folivore (e.g., Foley, 1987). Furthermore, such allelochemicals need to be detoxified before being excreted and detoxification is an energetically expensive process (Freeland and Janzen, 1974; Freeland and Winter, 1975).

The ability of animals to overcome this low energy availability of foliage is related to body size. Gut capacity decreases with decreasing body size (Parra, 1978). However, the relative energy requirements for maintenance and locomotion may increase as body size decreases (Kleiber, 1961; Taylor *et al.*, 1970; Schmidt-Neilson, 1974). Therefore, the ratio of energy requirements to gut capacity generally increases with decreasing body size (Parra, 1978) which has led several authors to propose a critical body weight below which utilisation of foliage is not possible (e.g., Eisenberg, 1978; Kay and Hylander 1978; Parra 1978). Nevertheless, it is advantageous for arboreal mammals to be small, relative to ground dwelling herbivores, to enable optimum mobility in the tree canopy

(Eisenberg, 1978).

Milton (1978) distinguishes 'anatomical' and 'behavioural' folivores according to the strategies used by vertebrates to overcome the above constraints and maximise the extraction of nutrients and energy from leaves. 'Anatomical' folivores (e.g., *Avahi laniger*, *Lepilemur mustelinus*, *Phascolarctos cinereus*, *Petauroides volans*) are characterised by physiological and anatomical specialisations of the digestive tract which allows them to efficiently utilise the abundant mature foliage which is frequently higher in fibre and allelochemicals, and possibly lower in protein than younger foliage (Feeny, 1970; Hladik A., 1978; Leigh and Smythe, 1978; Milton, 1978; Cork and Pahl, 1984). Such specialisations include either an enlarged foregut (e.g., *Giraffa camelopardalis*, *Bradypus variegatus*, *Colobus polykomas*,) or enlarged hindgut (e.g., *Elephas maximus*, *Lepilemur leucopus*, *Phascolarctos cinereus*, *Petauroides volans*) which increases the size of the fermentative pool in which energy can be liberated from cell walls by microbial digestion (Hladik C.M., 1978; Milton, 1978; Parra, 1978; Hume, 1982; Cork and Hume, 1983). For example, two marsupial folivores, *Petauroides volans* and *Phascolarctos cinereus*, have an enlarged caecum and an enlarged caecum/proximal colon, respectively (Hume, 1982; Cork and Hume, 1983). Studies on the digestive physiology of these species have found that they selectively retain the fluid and fine particles of digesta in the caecum and/or proximal colon while excreting the coarse particles of digesta (Hume, 1982; Cork and Warner, 1983; Foley and Hume, 1987a). It is postulated that this reduces the gut-filling effect of dietary fibre, thus enabling a higher rate of intake of the more digestible dietary constituents (Cork *et al.*, 1983; Cork and Warner, 1983; Cork and Sanson, 1990), and that it reduces the loss of microbial protein (Hume, 1982). The feeding behaviours of the 'anatomical' folivores reflect their digestive specialisations in that they tend to feed on foliage of only one or two tree species (Marples, 1973; Hladik, C.M., 1978; Hindell *et al.*, 1985; Martin 1985). In contrast, the digestive system of 'behavioural' folivores (e.g., *Alouatta palliata*, *Trichosurus vulpecula*) is less specialised and they rely more on selection of foods low in fibre and high in available energy and/or nitrogen (e.g., young leaves, fruits or animal tissues) to maximise their nutrient intake (Freeland and Winter, 1975; Milton, 1978; Parra, 1978; Hume, 1982; Kerle, 1984; Foley and Hume, 1987a,b).

The common ringtail possum, *Pseudocheirus peregrinus* (Boddaert, 1785)

(a member of the family Pseudocheiridae) is the smallest folivorous marsupial in Australia and it is also smaller than many eutherian folivores (e.g., see McNab, 1978; Clutton-Brock and Harvey, 1977). However, it appears to overcome the limitations of its small size, in part, by combining characteristics of both 'anatomical' and 'behavioural' strategies (Pahl, 1987b). The ringtail possum's digestive tract is similar to that of its close relative *Petauroides volans* (Hume, 1982) but the ringtail possum possesses a further digestive adaptation, i.e., caecotrophy (Tyndale-Biscoe, 1973; Chilcott and Hume, 1984). The ringtail possum is the only one of the folivorous marsupial species studied to date which combines selective fluid retention with caecotrophy (Chilcott and Hume 1985). This reduces the ringtail possum's dietary nitrogen requirement by 50% (Chilcott and Hume, 1985). In addition, Foley (1987) notes that, on the basis of work on *Lepus timidus* (Pehson 1983) which consumes foods high in fibre, caecotrophy in the ringtail may lead to a significant increase in cell-wall digestibility, which may contribute to the relatively fast rate of digesta passage recorded in the ringtail possum (Chilcott and Hume 1985). Such specialisations enable the ringtail possum to utilise a diet generally low in nitrogen and high in dietary fibre (Chilcott and Hume 1984a; Cork and Pahl, 1984; Pahl, 1987b). Furthermore, the behavioural feeding strategies of the ringtail possum, that is, food selection at the level of species, tree, leaf and leaf part, may increase its intake of high quality food (Pahl, 1987b).

Arboreal folivores appear to share 'energy-saving' strategies, in addition to strategies which maximise energy and nutrient intake from their poor quality diet. These include reduced activity patterns (MacLennan, 1984), small home range (Kehl and Borsboom, 1984), and the use of gliding as a form of locomotion (Eisenberg, 1978). Heat conserving mechanisms also appear to predominate particularly amongst the small folivores. Such mechanisms may include the development of a dense pelage (Eisenberg, 1978) or nest building thus creating a small microclimate (e.g., *Lepilemur leucopus*, Klopfer and Boskoff, 1979). The coevolution of such strategies has led to the suggestion that folivorous mammals in general, are adapted to a lower energy requirement than mammals feeding on high quality diets (Eisenberg, 1978). McNab (1978, 1986a) concludes that a reduced basal rate of metabolism may be a general feature of arboreal folivores. However, Hume *et al.*, (1984) argue that a low total energy requirement for free existence

(field metabolic rate) rather than basal metabolic rate is a characteristic of folivorous mammals. Slow rates of growth, reduced litter size and hence low offspring production rates also appear to be characteristics shared by many arboreal folivores (McNab, 1980, 1983; Russell, 1982; Smith and Lee, 1984). The question that arises, therefore, is does the 'energy-conserving' hypothesis extend to reproduction?

In eutherian mammals the energy requirements of lactation generally exceed the requirements of other reproductive events such as mating, gestation, and post-lactational parental care (Hanwell and Peaker, 1977; Randolph *et al.*, 1977; Stebbins, 1977 Oftedal, 1985; Gittleman and Thompson, 1988). In contrast to most eutherian young, only a small proportion of the developmental period of marsupial young is spent in utero and birth weight in marsupials is lower than in any eutherian of a comparable body weight (Loudon, 1987). Clearly, marsupials differ from eutherians in that nutrient transfer from the mother to the young occurs predominantly during lactation, whereas nutrient transfer in eutherians is divided, to varying extents, between gestation and lactation (Tyndale-Biscoe and Renfree, 1987). This difference in mode of reproduction, and the lower metabolic rate of marsupials compared to eutherians (Dawson and Hulbert, 1970; Kinnear and Shield, 1975), has led several authors to suggest that reproduction would be less efficient and more energetically costly in marsupials than in other mammals (Lillegraven, 1975; Lillegraven *et al.*, 1987). Although only some components of maternal energy expenditure during reproduction have been investigated in marsupials (Green, 1984; Thompson and Nicoll, 1986; Loudon, 1987; Rose, 1987; Green *et al.*, 1988), the little data available does not appear to support such speculation. In addition, one recent study on the tammar wallaby, *Macropus eugenii*, suggests that the overall metabolisable energy requirement for reproduction is similar for both eutherian and marsupial herbivores (Cork and Dove, 1989).

Maternal milk must nourish the young through a series of anatomical and physiological changes, transmitting passive immunity, supporting growth of intestinal flora and providing energy (Jenness and Sloan, 1970). The compositional changes that occur throughout lactation in marsupial milk appear to reflect the growth and developmental stages that the young goes through, during this period of its life (Green, 1984). Tyndale-Biscoe and Janssens (1988) divided the marsupial lactation cycle into three 'Phases' to facilitate comparison between species. Phase one occurs during pregnancy when the mammary gland develops the capacity for milk synthesis. The second Phase commences with the birth of the young, when

initiation and maintenance of lactation occurs. The third and final Phase begins when the young vacate the pouch or are left in the nest and ends when the young are weaned.

Adaptive significance has been attributed to differences in the lactational strategy (i.e., length of lactation, milk composition and milk production) both between and within groups of eutherian mammals (Ben Shaul, 1962; Jenness and Sloan 1970; Maltz and Shkolnik, 1984; Oftedal, 1984; Gittleman and Oftedal, 1987). For example, Gittleman and Oftedal (1987) found that amongst the Carnivora, species with herbivorous/folivorous diets have slower growth rates and lower peak milk energy outputs than do more carnivorous species. They suggest that a diet low in available energy may constrain nutrient transfer rates from the mother to the young. A similar adaptive reasoning has been made by Eisenberg (1981) for the slow growth rate of the elephant, *Elephas maximus*.

Differences in milk composition and length of lactation between marsupial species studied to date also suggest adaptive constraints (Merchant *et al.*, 1989). However, there have been no studies on marsupial folivores. Nevertheless, although the mass of one young at weaning as a percentage of the maternal body weight does not differ greatly between phalangeroid, dasyurid or macropodoid marsupials the length of time to weaning does, being longest in the herbivorous/folivorous species (Russell 1982; Smith and Lee, 1984). Russell (1982) proposes that this is because leaves are less nutritious, therefore, only a low metabolic rate can be maintained by the mother and hence a low rate of milk production.

Many herbivores appear to overcome the problem of increased energy and nutrient requirements associated with reproduction by timing the most expensive phase to coincide with periods of increased food quantity or quality (Viljoen, 1981; Sadleir, 1969). Studies on the ecology and population biology of the ringtail possum are confined to those inhabiting areas in Victoria and New South Wales (Thompson and Owen, 1964; Marsh, 1968; Hird, 1975; How *et al.*, 1984; Pahl and Lee, 1988). These studies have shown that the ringtail possum is a partly seasonal, partly opportunistic breeder. It is surprisingly fecund producing between one to three young a year (Thompson and Owen, 1964; Pahl and Lee, 1988) compared with the single offspring produced by other folivorous marsupials (e.g., *Trichosurus vulpecula*, *Petauroides volans*, *Phascolarctos cinereus*). There are also

variations in fecundity both within and between populations. Some of these variations in timing of reproduction and fecundity are related to the age structure and weight of the females in the population (Pahl and Lee, 1988). However, information on the pattern of energy allocation by a ringtail possum throughout its annual cycle would enable clarification of the circumstances leading to seasonal breeding and any individual variation in fecundity (e.g., Kenagy *et al.*, 1989). Furthermore, the efficiencies and absolute rates of use of energy and water for reproduction are potentially subject to natural selection, hence their assessment would provide an idea of an animal's evolutionary fitness (Kenagy, 1987).

Aims and Organisation of the Thesis.

The objectives of this study were threefold. Firstly, to determine the annual cycle of energy expenditure by adult free-living common ringtail possums, *Pseudocheirus peregrinus*, in particular, the energy expended in lactation by the females. Secondly, to detail the lactational strategy and growth of the common ringtail possum. Thirdly, to quantify the total allocation of time and energy to lactation and the strategies employed by the female to cope with the additional energy requirements of lactation.

The thesis may be divided into three sections. The first deals with the annual timing of reproductive events, growth and aspects of the population biology of the animals studied. In addition, their activity patterns are described and a qualitative assessment of the diet is given. The second addresses the energetics of the free-living animals and the third examines the lactational strategy (milk composition and production).

CHAPTER 2

DISTRIBUTION, DESCRIPTION OF THE STUDY AREA AND GENERAL METHODS

2.1 Distribution of *Pseudocheirus peregrinus* in Tasmania

The common ringtail possum, *Pseudocheirus peregrinus* is distributed throughout the east, south-east and south-west of Australia. There are five distinct subspecies, with the animals found in Tasmania and the Bass Strait islands being known as *Pseudocheirus peregrinus viverrinus* (Ogilby, 1837). The Tasmanian common ringtail possum was first described by William Anderson (1777) who was a member of Cook's third voyage in 1776 (Stanbury, 1978; McKay, 1989). Prior to 1940 the common ringtail possum appears to have been particularly numerous throughout Tasmania and records indicate that between 1923 and 1955 7,500,000 animals were hunted for their commercially valuable fur (Guiler, 1957). However, the common ringtail possum (hereafter referred to as the ringtail possum) population on the Tasmanian mainland declined rapidly after 1940 and numbers only started to recover in 1963-64 (Guiler, 1967; Green, 1973). The ringtail possum is now a wholly protected species in Tasmania (National Parks and Wildlife Act, 1970).

Information on the present distribution of the ringtail possum in Tasmania was collected from four sources (Figure 2.1) i.e.:

- (1) occupied ringtail possum drey (nest) sightings (personal observations; Nigel Brothers, personal communication);
- (2) spotlighting and road-killed animals (personal observations; Nigel Brothers, personal communication);
- (3) museum specimens and published reports (Slater, 1987; Green, 1973);
- (4) computerised records of animal distributions in Tasmania ("TASPAWS", National Parks and Wildlife Service).

This information suggests that the ringtail possum is found throughout Tasmania in a variety of tree communities (see Figure 2.1). Ringtail possum populations inhabiting forests in New South Wales (Braithwaite *et al.*, 1983) and Victoria (Pahl, 1984) show differences in densities between various plant associations. Preliminary estimates of densities of ringtail possum populations in various forest communities throughout the state suggest that a similar situation

exists for Tasmanian ringtail populations (personal observation)

Amongst the islands of the Bass Strait, ringtail possums are known to be present on Flinders Island, Cape Barren Island and King Island (Hope, 1969). However, it is possible that they may inhabit some of the other islands such as Prime Seal Island, Three Hummock Island and Hunter Island where there is suitable habitat and few detailed mammal surveys have been made.

2.2 Study Area

The choice of study area was based on two criteria:

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Although the area around the study site on Flinders Island had been cleared for pasture, the site itself had been completely unaffected by fire or clearing at least since 1920 (D. Smith, personal communication) and almost certainly much longer. Coast teatree, *Leptospermum laevigatum* dominated the study area. This species is a tall shrub or small tree of 6-12m high with extensive branching (see Curtis and Morris, 1981). Within the study area coast teatree varied from dense stands of single-stemmed trees of 6-8m high with diameter at breast height (D.B.H) of about 13 cm forming a closed canopy, to more open stands of tall (12m) and multi-stemmed trees (D.B.H = 30 cm) with gaps in the canopy. In the latter stands, old dead shrubs were common. This subjective assessment suggests that the study area is an uneven-aged stand of coast tea-tree with similar old stands to those noted in Victoria by Hazard and Parsons (1977).


Coexisting tree species in the study area were *Casuarina stricta*, *Callitris rhomboidea*, *Melaleuca ericifolia*, *Acacia sophorae* and one individual *Eucalyptus globulus*. Shrubs/small trees of *Leucopogon parviflorus*, *Myoporum insulare* and *Acacia mucronata* were recorded in the understory. Where the teatree canopy was open *Olearia axillaris* and *Boronia heteronema* were recorded as ground cover.


Figure 2.1 Distribution of the common ringtail possum, *Pseudocheirus peregrinus viverrinus* in Tasmania (personal observations; N. Brothers, personal communication; Green, 1973; Slater, 1987; "Taspaws", National Parks and Wildlife Service).


● = locations within a 10° grid.


Vegetation map derived from Kirkpatrick and Dickenson (1984).


 = Rainforest, blackwood swamp forest and mixed/wet sclerophyll forest.

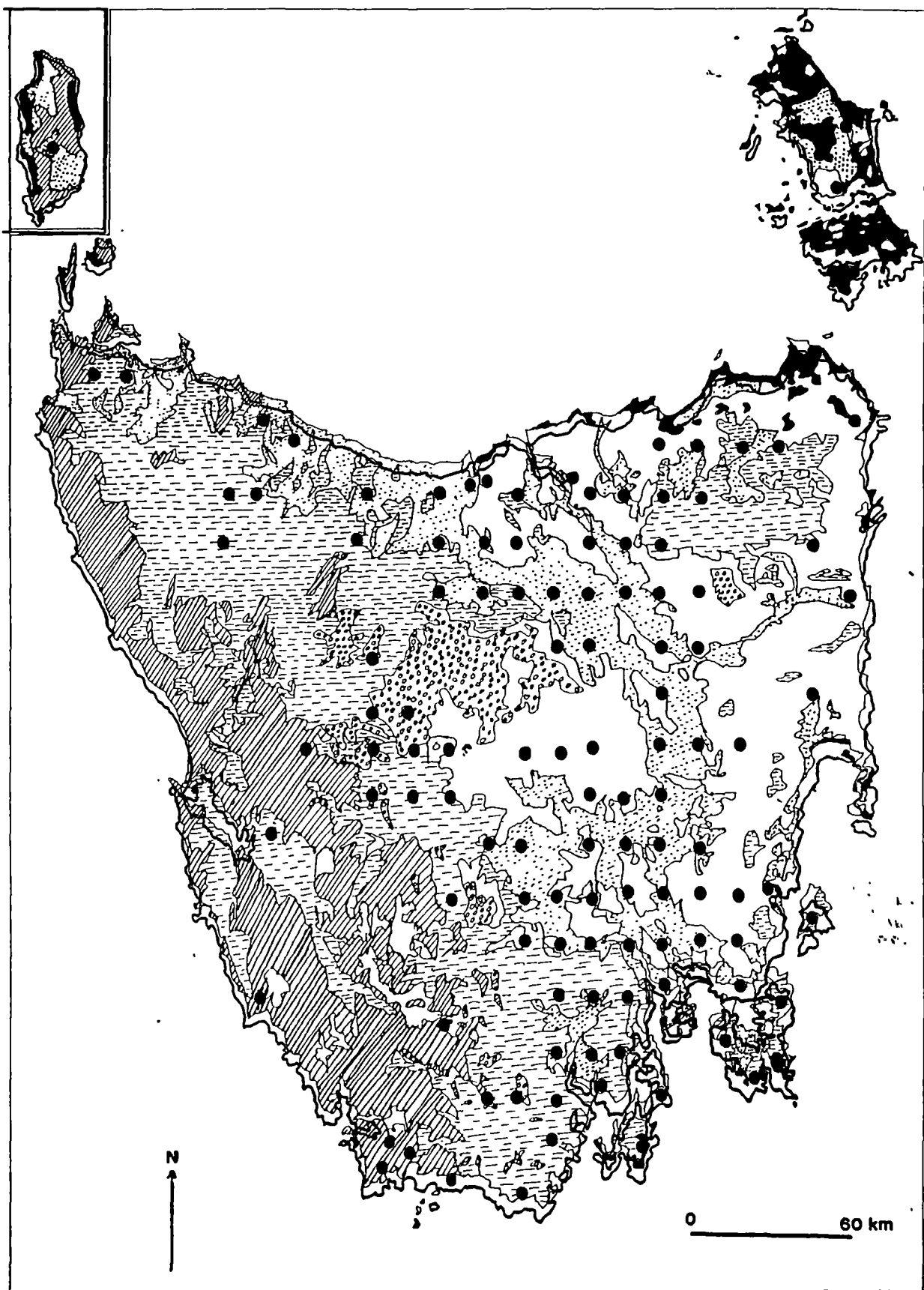
 = Dry sclerophyll/alpine mosaic and alpine vegetation.

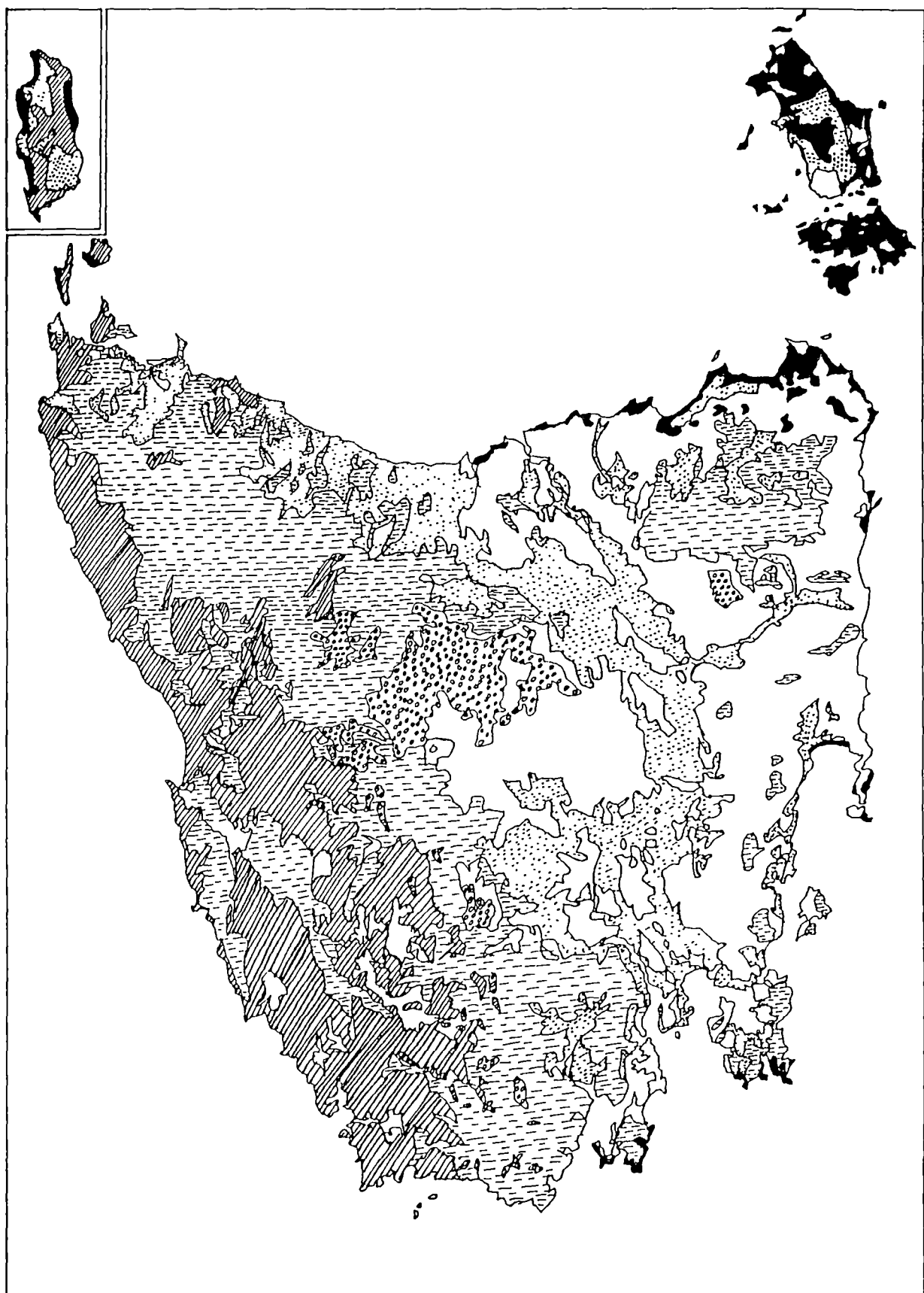
 = Wet sclerophyll/dry sclerophyll mosaic, dry sclerophyll forest and woodland.












 = Dry coastal vegetation.

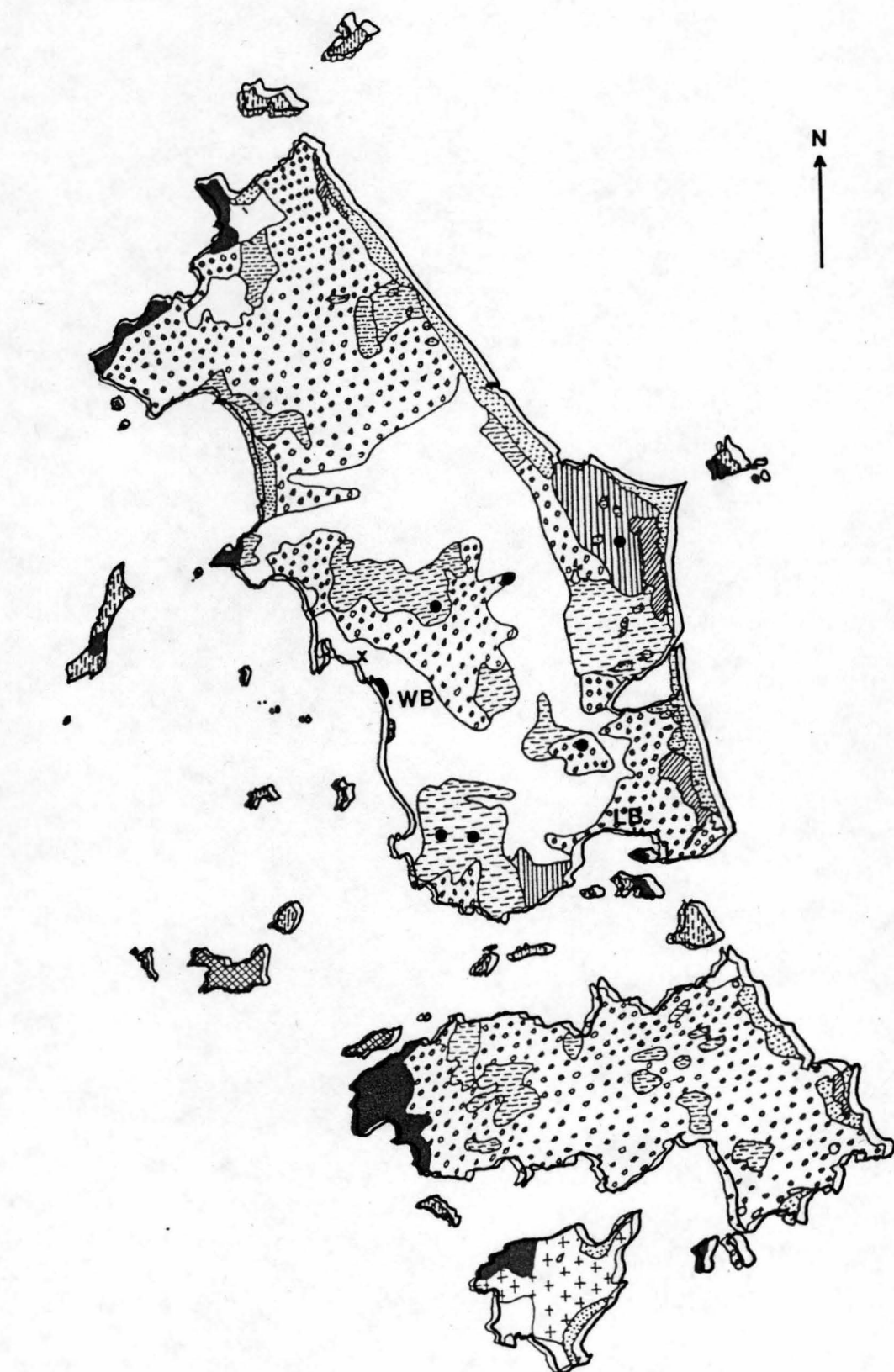
 = Moorland and scrub.

 = Native grassland and cleared land (as at 1960).

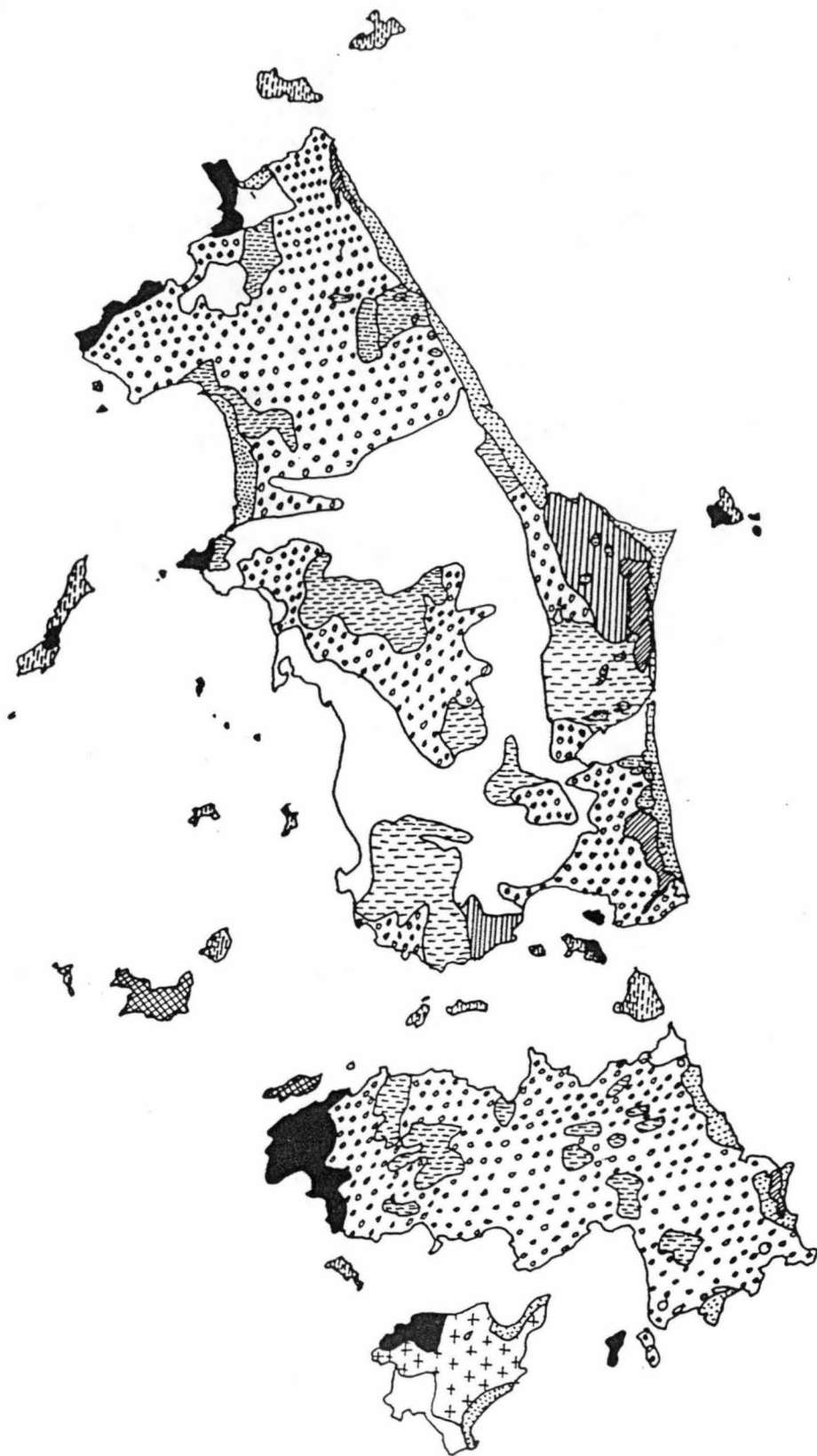




- Figure 2.2** Distribution of the common ringtail possum, *Pseudocheirus peregrinus viverrinus* on Flinders island and location of the study area.
- = location of known ringtail possum populations.
 - ▲ = location of study site.
 - W = Whitemark.
 - LB = Lady Barron.
 - X = Weather station at airport.
- Vegetation derived from Kirkpatrick and Dickenson (1984).
-  = Scrub/heath mosaic.
 -  = Sclerophyll forest.
 -  = Scrub
 -  = *Casuarina stricta* forest.
 -  = Coastal complex.
 -  = Cleared land.
 -  = Saline wetland.
 -  = Heath.
 -  = Fresh wetland.
 -  = Island complex.
 -  = Grassland.



0 10 km



exists for Tasmanian ringtail populations (personal observation)

Amongst the islands of the Bass Strait, ringtail possums are known to be present on Flinders Island, Cape Barren Island and King Island (Hope, 1969). However, it is possible that they may inhabit some of the other islands such as Prime Seal Island, Three Hummock Island and Hunter Island where there is suitable habitat and few detailed mammal surveys have been made.

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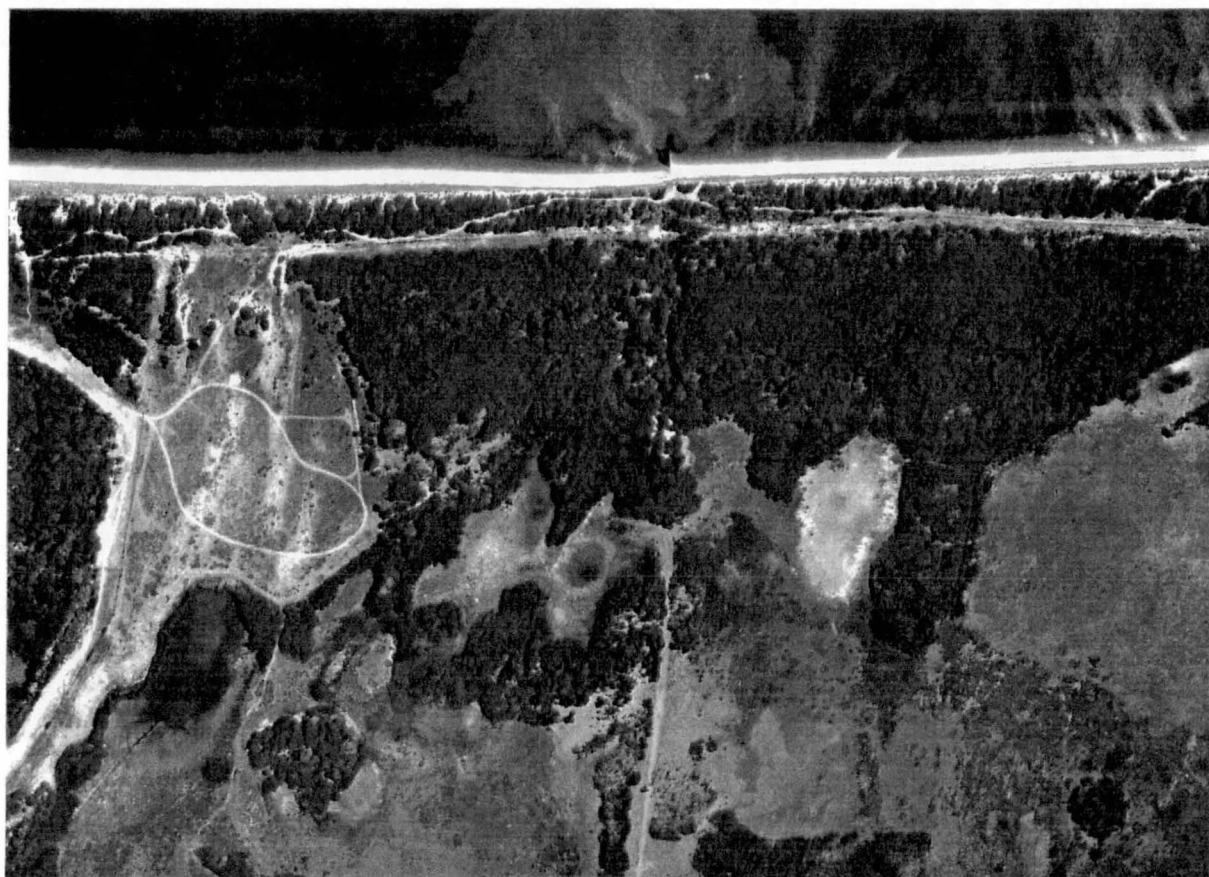


Figure 2.3 Whitemark Beach region (a) and Paddies region (b) of the study site on Flinders island.

Only a few swamp paperbarks, *Melaleuca ericifolia*, were found amongst the teatree stands however this species dominated around the lagoon and on the swampy inland ground (Figure 2.3).

Flowering and fruiting of *L. laevigatum* and *M. ericifolia* was observed during the spring and early summer months. The production of young leaves was also noted during the spring and summer months.

Percentage canopy cover of each tree species and the percentage number of each tree and shrub species in 1 hectare were used as measures of species abundance (Table 2.1). Ten transects (10m x 50m) were randomly set out throughout the study area. The mean canopy cover for each species in each transect was estimated from a sample of 8-15 individual trees by means of the equation;

$$A = \pi r^2,$$

where A = area of canopy cover and r is the average of the maximum and minimum canopy radius in metres (Pahl, 1987b). An estimate of the canopy cover of each tree species in each transect was then estimated as the product of the number of trees of each species and the mean canopy cover for that species. Estimates for each transect were then combined to give a percentage canopy cover estimate for each species. Counts of a particular tree species (including understory species) were also combined and expressed as a percentage of the total number of trees counted (Table 2.1)

The study area was divided into two regions, by the small town of Whitemark. One region, 'Whitemark Beach' (17.5 hectares), was situated north of Whitemark. The second region, 'Paddies' (15 hectares), was situated south of Whitemark (Fig. 2.2 and Fig. 2.3). Movement of ringtail possums between these two regions was known to occur. Each study region was bound to the east by grazing pasture and to the west by Parrys Bay. There was little disturbance by man during the two year study and interested local residents helped to supervise the area.

The ringtail possums' nests or dreys were found predominantly in the branches of *L. laevigatum* although nests were also located in other tree species including the forked branches of the *E. globulus* and in the understory bushes of *L. parviflorus*.

Ringtail possums were found in several other areas on Flinders island (see Figure 2.2). Early in the study animals were sampled in Strzelecki National Park. However, capture of the ringtail possums, by hand (see later), proved to be

Table 2.1 Percentage canopy cover and abundance of tree species in the study area.

Tree Species	Canopy cover (%)	Species abundance (%)
<i>Leptospermum laevigatum</i>	74.6	76.4
<i>Casuarina stricta</i>	18.9	4.3
<i>Melaleuca ericifolia</i>	5.1	11.3
<i>Callitris rhomboidea</i>	1.1	1.6
<i>Acacia sophorae</i>	0.9	0.8
<i>Leucopogon parviflorus</i>	(understory)	5.5
<i>Eucalyptus globulus</i>	-	0.03

particularly difficult at Strzelecki due to the nature of the tree community (open-forest dominated by *E.nitida* (Kirkpatrick and Dickenson, 1984), therefore this site was abandoned.

Other mammal species known to inhabit the study area included *Vombatus ursinus*, *Thylogale billardierii*, *Macropus rufrogriseus rufrogriseus*, *Tachyglossus aculeatus setosus*, *Trichosurus vulpecula* and *Felis catus*.

Feral cats in the area were found to predate on young ringtail possums. Ringtail possums were also probably taken by raptors. Wedge-tailed eagles (*Aquila audax*), brown falcons (*Falco berigora*), the grey goshawk (*Accipiter novaehollandiae*) and the masked owl (*Tyto novaehollandiae*) are all known to consume ringtail possums on mainland Tasmania (N.Mooney, personal communication; Green, 1982).

2.3 Climate

Daily rainfall and temperature information for the study area was obtained from the Bureau of Meteorology weather station situated at Whitemark airport (approx. 1km north of the study area, see Figure 2.2). Monthly maximum and minimum temperatures and monthly rainfall measurements are presented in Figure 2.4. Measurements were also obtained for the days over which the study was conducted and these are presented in Figure 2.5. The climate on Flinders Island may be described as cool temperate maritime, characterised by cool winters and warmer summers.

The thermoneutral zone for *P.peregrinus viverrinus* (see Chapter 5) is illustrated on Figure 2.4 and Figure 2.5. During the winter months the maximum daily air temperature in the shade was within 10°C of thermoneutrality, however the lower minimum temperatures may have an impact on the energy expenditure of the ringtail possum (see Chapter 6).

Sunset and sunrise times for Flinders Island were also obtained from the Bureau of Meteorology. The mean times for 1986-1988 are presented in table 2.4.

2.4 Field Methods

2.4.1 Catching programme

The ringtail possum population was examined by capturing individuals by

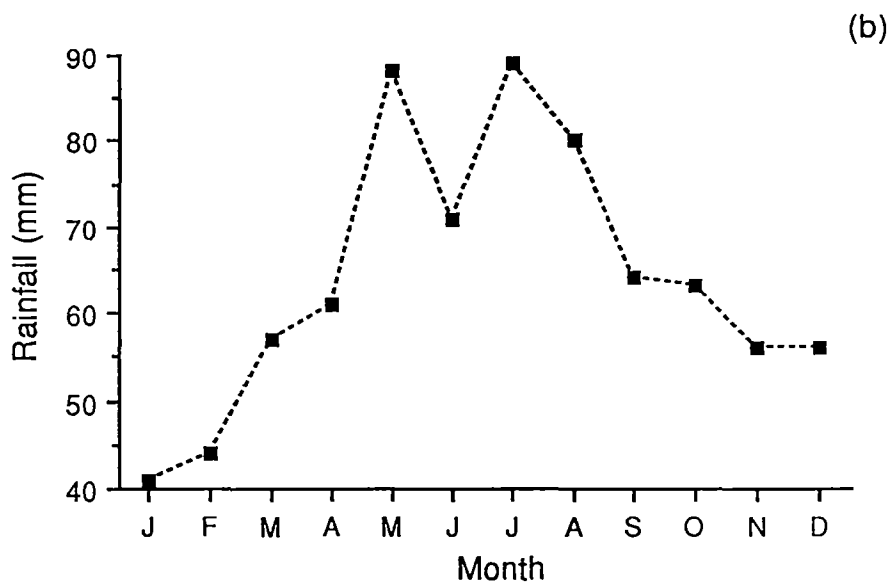
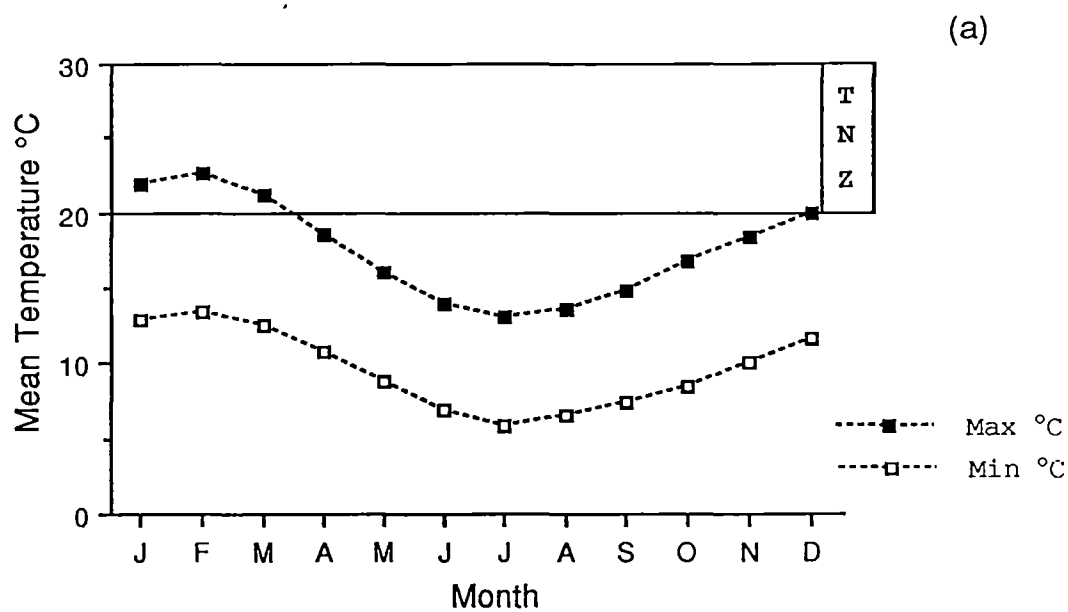


Figure 2.4 Mean monthly temperature (a) and mean monthly rainfall (b) for Flinders island, Bass Strait. **TNZ** = Thermoneutral zone for *Pseudocheirus peregrinus viverrinus* (see Chapter 5).

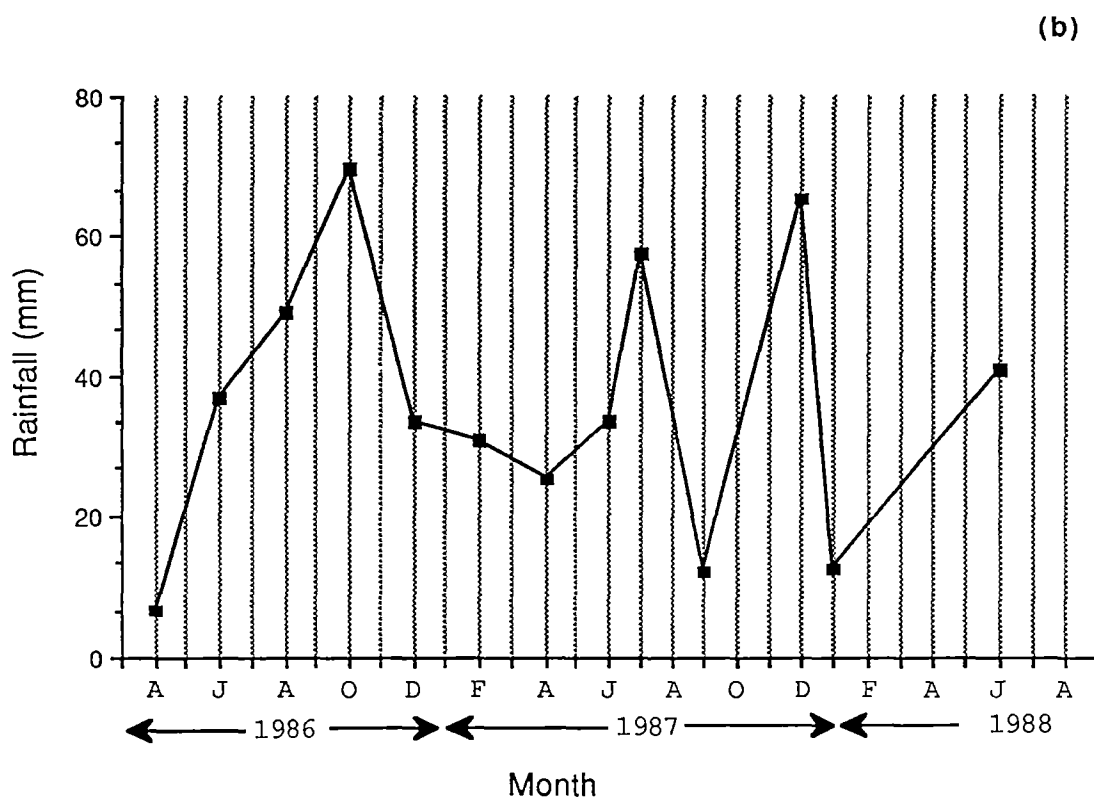
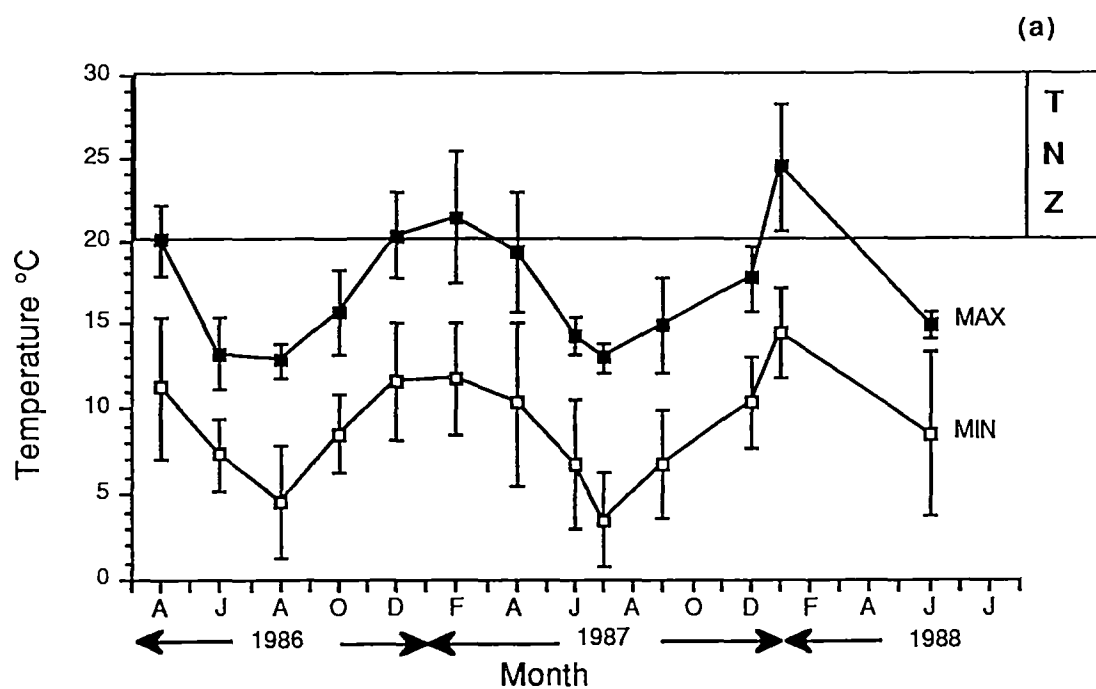


Figure 2.5 (a) Mean (\pm SD) ambient temp and (b) mean rainfall for the days over which the study was conducted. TNZ = thermoneutral zone of the ringtail possum (see Chapter 5).

Table 2.4 Mean sunset and sunrise times for 1986 - 1988 on Flinders Island.

Month	Sunset (hrs and mins)	Sunrise (hrs and mins)
January	20:38	6:08
February	20:14	6:34
April	17:60	6:28
June	16:44	7:43
July	17:07	7:27
August	17:31	N/D*
September	17:58	6:14
October	19:52	5:57
November	20:26	5:29
December	20:39	5:25

* N/D = no data

hand. The nests (dreys) built by the ringtail possums were located during the day and the tree or bush containing the nest was marked with red surveyors tape and a numbered metal tag. The tree containing the nest was then gently shaken and any animals which emerged from the nest were encouraged to move away from the nest tree by further shaking of nearby trees. When the possum reached a suitable tree or bush, it was dislodged by shaking the tree vigorously and then caught by hand. This technique has been used successfully in studies on other ringtail possum populations (e.g., Thompson and Owen, 1964; Hird, 1975; How *et al.*, 1984; Pahl, 1987a). Although often time consuming, especially in areas of tall mature teatree, this method of capture has several advantages over cage trapping (discussed by Thompson and Owen, 1964). In particular, when virtually all the nest sites in an area are known, an idea of movements or home range of a particular possum may be obtained from successive captures. Additionally, information on social grouping of the possums may be obtained from successive captures (see Chapter 4). A third advantage relevant to this particular study relates to the use of the doubly labelled water technique to measure energy expenditure (see Chapter 6). This technique depends upon the recapture of a particular individual after a specific period of time. Location of individuals in their dreys during the day with the help of radiotransmitters (see Chapter 4) and subsequent capture by hand assured recapture whereas cage trapping was not successful.

Field trips were made between April 1986 and July 1988 (see Table 2.2). All established nest sites (located on previous trips) in the study area were examined and new nests were located and recorded. The nest site and identity of individuals sharing a nest was noted at each capture. All individuals were released on the tree containing the nest immediately after handling.

2.4.2 Handling and measurements

After capture animals were placed in a calico bag. If unmarked the animals were tagged in one ear with a numbered 1cm fingerling fish tag (Saltlake City Stamp Co.). All animals were sexed and weighed with a Pesola (1.5kg) spring balance to the nearest 20g or a Super Salter (1kg) spring balance to the nearest 10g. These balances were periodically checked for accuracy against a known standard. The body mass (g) of females carrying pouch young did not include the weight of the young. If the young were too small to remove from the pouch before weighing their

Table 2.2 Schedule of fieldtrips.

YEAR	MONTH	DAYS
1986	April	10 th - 20 th
"	June	9 th - 22 nd
"	August	12 th - 24 th
"	October	19 th - 28 th
"	December	3 rd - 17 th
1987	February	9 th - 23 rd
"	March/April	28 th - 13 th
"	May/June	25 th - 8 th
"	July	18 th - 31 st
"	September	7 th - 21 st
"	November/December	22 nd - 8 th
1988	January/February	18 st - 2 nd
"	June/July	22 nd - 10 th

weight was estimated from the age weight relationship for captive animals (see Chapter 3) and subtracted from the mother plus litter weight. Headlength, pes length, and leg length were measured to the nearest 1mm with calipers and tail length and white section of the tail were measured to the nearest 1mm with a plastic tape (see Fig. 2.6). Observations were made of the animals external condition, pelage colour (underfur either grey, rufous or ginger; guard hairs either brown, rufous, ginger or black) and any distinguishing features (e.g., ear tears, position of white tail tip). This enabled identification of individuals upon capture if loss of tags occurred. If ticks were found on an animal these were removed and placed in vials containing alcohol for later identification (see Appendix A).

The reproductive status of captured animals was noted (see Chapter 3 for details). The testes of male possums were measured externally through the scrotum using calipers. The length and the width of the right testis was measured to the nearest 1mm. The degree of invagination of the pouch and condition of the skin lining the pouch (i.e., dry, moist red/brown secretion or scaly) were noted. The nipples in the pouch were described as either inverted or everted and the pouch entrance was recorded as either loose or constricted. Any pouch young were counted and sexed when possible. Measurements of pouch young were made following Lyne and Verhagen (1957). Headlength was the only measurement made on young attached to the teat. Details of other measurements made on suckling young and developmental features noted are given in Chapter 3.

2.5 Ringtail Possums in Captivity

2.5.1 Collection, maintenance and release site

Eight female ringtail possums and four males were captured by hand from areas other than the study area on Flinders island. These animals were transported to the University of Tasmania in Hobart within a day of capture and placed in large outdoor enclosures.

Groups of animals caught from the same nest in the wild were placed together in individual enclosures. The most successful combinations of animals held in the same enclosure were male/female and female/female pairs. Males held together, or more than one female held with a single male were found to fight, inflicting wounds on the opponents hindquarters, base of tail and ears.

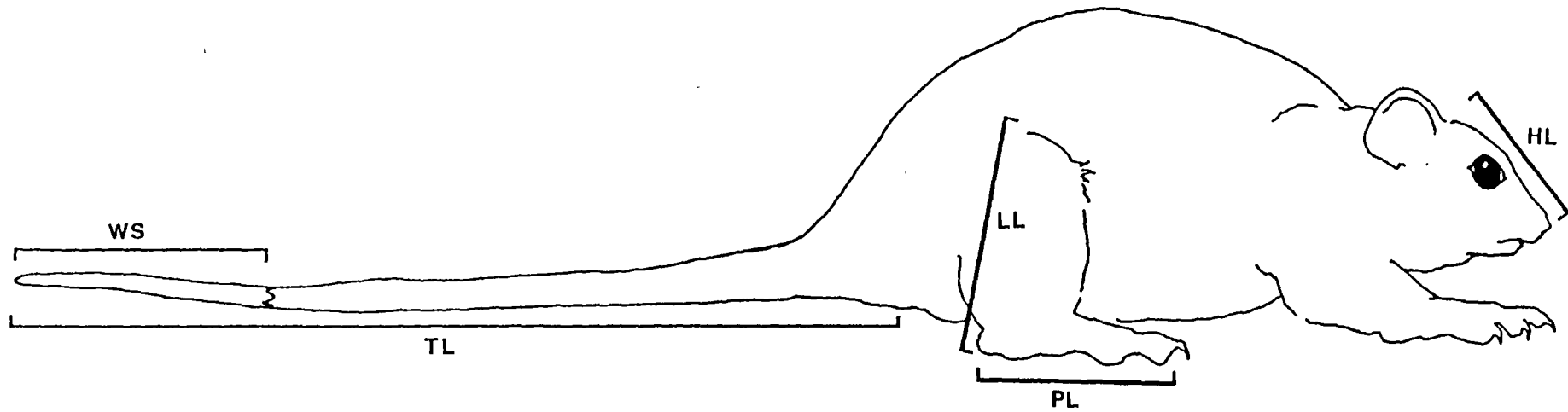


Figure 2.6

Illustration of the measurements taken for an adult ringtail possum. HL = head length, PL = pes length, TL = tail length, WS = white section of the tail, LL = leg length.

Each enclosure was equipped with large climbing branches and two nest boxes (see Fig 2.7). Foliage collected each day from *Eucalyptus amygdalina* saplings located on Mount Nelson, Hobart and apples were placed in each cage during the late afternoon. Fresh water was also supplied daily. A mixture of peanut butter/honey and oats was offered on occasions.

Within a month of capture and transfer to captivity all but one possum had adapted to the new diet and were easily handled. The one animal which did not maintain weight was blind in one eye and in poor condition when caught. This female was released at the site of capture six months after being brought into captivity.

Captive animals were weighed and their reproductive status was noted each week (except during fieldtrips) as described above for field animals. All the females placed with a male mated and successfully reared young. Details of the animals maintained in captivity and their offspring are given in Table 2.3. Family groups remained together in one cage without aggressive interactions, even when the offspring had reached maturity.

At the end of the study the animals and their offspring were released on the east coast of Flinders island in an area which had few resident possums due to a wildfire in 1979. Subsequent trips to the island revealed that these animals have become established in the wild and are breeding successfully.

2.5.2 Deaths and diseases in captivity

Eight animals died in captivity. One was a juvenile male born in captivity who was found to have multiple stomach ulcers. These were thought to be due to stress caused by a feral cat found near to the enclosures. The second was an adult female who died of suspected toxoplasmosis. The third was an old adult male who had severely worn teeth. This male probably could not effectively chew his food which may have contributed to his loss of weight and ultimate death. A similar scenario was suggested for an old male in the wild by Pahl (1987a). The remaining five animals which died were five pouch young (from three litters).

Alopecia (particularly around the eyes and base of the tail), loss of body weight and general condition was noted in one old male. This was consistent with irritation caused by a mite infestation (Presidente, 1982). This mite spread to a female suckling two pouch young who shared the cage with the infested male. An

Table 2.3 Animals maintained in captivity.

Female	Male Mate		Offspring	
	1986	1987	1986	1987
F1	M3	M3	A,B	H,I
F2*			S,T	
F3	M4	-	C,D	-
F4	M1	M1	E	O,N
F5	M2	M2	F,G	L,M
F6	-	M4	-	K,J
F7	-	M2	-	P
F8	-	-	-	-
A		T		Q,R

* mated in the wild



Figure 2.7 Enclosure for animals in captivity.

insecticidal wash and removal of the infested male helped to control the condition in the female. However, her lower condition was reflected in the slow rate of growth of her young (see Chapter 3).

Loss of hair, particularly around the base of the tail, was apparent in some animals caught in the wild (see Figure 2.8). This suggests that wild animals also suffer from occasional mite infestations.



Figure 2.8 Loss of hair around the base of the tail and rump of a male ringtail possum caught in the wild.

CHAPTER 3

GROWTH AND REPRODUCTION OF THE COMMON RINGTAIL POSSUM IN TASMANIA.

3.1 Introduction

A detailed study of the reproductive biology of the female ringtail possum found that it was both polyoestrous and polyovular (Hughes *et al.*, 1965). Studies of wild ringtail possums in Victoria and New South Wales show variations in the duration of the breeding season and rates of fecundity both within and between populations (Thompson and Owen 1964; Marsh 1968; Hird 1975; How *et al.*, 1984; Pahl, 1985). Pahl and Lee (1988) relate some of these differences to the age structure and weight of females in the population. They conclude that the reproductive strategy of the common ringtail possum is partly seasonal and partly opportunistic. The main breeding season was during the autumn/winter months, in all populations studied, so that late lactation coincided with increased leaf production in late spring.

There have been no detailed studies of reproduction in wild populations of the ringtail possum, *P. peregrinus viverrinus* in Tasmania. However, Flynn (1922) recorded breeding, by *P. peregrinus viverrinus*, from May to August and suggested births may also occur during the summer months. In addition, anecdotal records indicate that the breeding season in Tasmania may be similar to Victorian populations (R. Green, personal communication; Green, 1973).

Smith and Lee (1984) showed that folivorous marsupials have low 'offspring production rates' (product of litter size and weight of one young at weaning divided by the time to weaning). They argue that the low proportion of protein in leaves and the low absolute energy intake of arboreal folivores (McNab, 1980) may affect milk quality and quantity and this could restrict the growth rate of the sucking young. Russell (1982) compared the growth rate of a range of marsupial species from pouch emergence to weaning. Amongst the arboreal folivores, she noted a particularly slow rate of growth only for the koala, *Phascolarctos cinereus*. Nevertheless, she did show that a long period of lactation and slow rate of development was a characteristic of the folivorous species compared with other

arboreal species.

Some studies note aspects of the growth and development of the common ringtail possum (Thompson and Owen, 1964; Presidente, 1982; Hird, 1975; How *et al.*, 1984; Pahl, 1987a). Thompson and Owen (1964) describe the pattern of growth during pouch life and detail the development of the young from birth to maturity in a wild population at Warramate in Victoria. In addition, the pattern of growth and development has been described for young in a wild population at Sandy Point in Victoria (Hird, 1975; How, 1984; Pahl, 1987a). However, no study has examined, in detail, the pattern of growth or growth rate of ringtail possum young from birth to maturity.

Parameters such as litter size, growth rate of the suckling, mass of the young at weaning and duration of lactation vary both within and between mammalian species (e.g., Russell, 1982). Therefore details of such parameters are required before effective intra- and inter-specific comparisons of energy expended during reproduction can be made (Lee and Cockburn, 1985; Gittleman and Thompson, 1988).

This study examined aspects of reproduction in a population of *Pseudocheirus peregrinus viverrinus* on Flinders Island and the pattern of growth, growth rate and rate of development of young in captivity and in the wild.

3.2 Methods

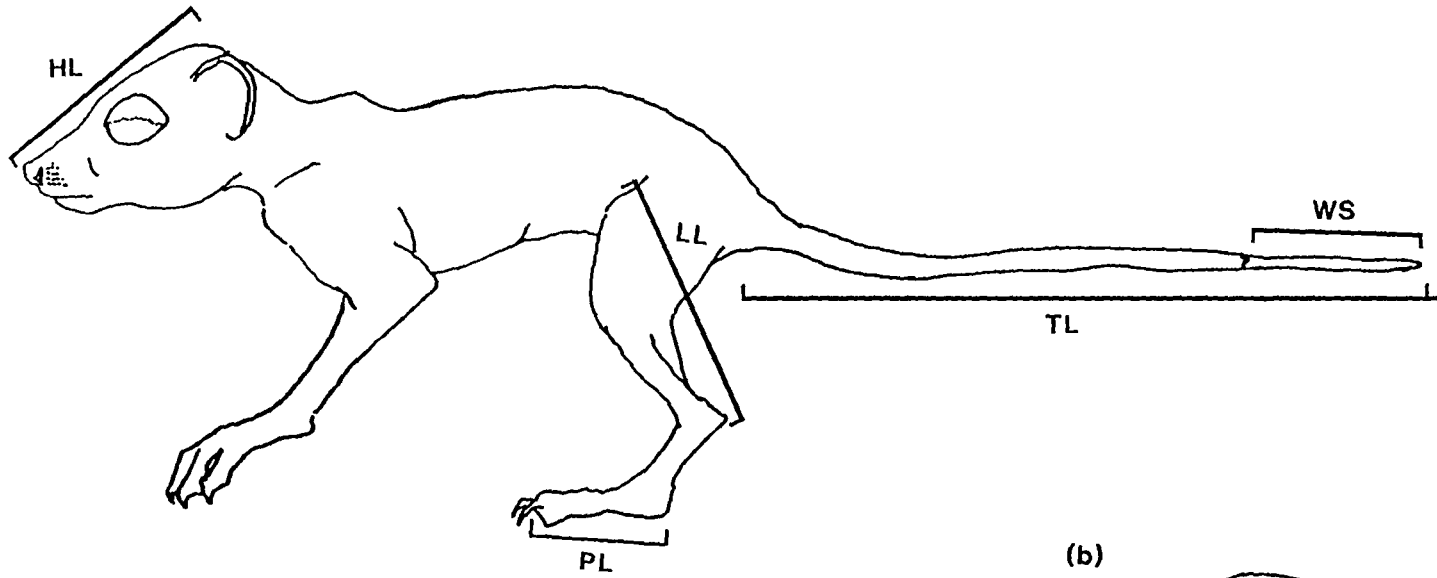
3.2.1 Study area and animals

The study area, general field techniques and measurements involved in the capture-mark-release programme have been described in Chapter 2. Details of animals maintained in captivity and their offspring are also given in Chapter 2.

3.2.2 Growth measurements

Growth data was obtained for eleven litters reared in captivity (total of twenty individuals, see Table 2.3, Chapter 2). The young were measured at weekly intervals from birth to weaning. Measurements were made on three animals from birth to maturity (A,S and T, see Chapter 2).

(a)



(b)



Figure 3.1

Diagram of measurements made on young ringtail possums.

Body measurements of the young were collected as illustrated in Figure 3.1 (adapted from Lyne and Verhagen, 1957). Measurements were made to the nearest 0.1 mm using vernier calipers. Tail length was measured to the nearest 1 mm using a plastic measuring tape. Body mass of young which had released the teat and could be easily removed from the pouch was measured to the nearest 0.01 g using a Mettler PE 3600 balance.

The number of young and the position of individuals in the pouch (i.e., right or left posterior teat) was noted. Sex of the young could be discerned between 17 and 52 days after birth, which enabled identification of individuals. However, if siblings were the same sex then a single tail nick made with a scalpel enabled them to be distinguished, until they were older and natural distinguishing features had developed (e.g., white pelage markings, manus prints).

In general, young caught in the field were measured as described for young in captivity. However, the mass of field young was measured using a pesola spring balance to the nearest 0.1 g and pouch young were not removed from the pouch until they were about 2 months old. Individuals were identified by their sex. Pouch young were not marked in the field, therefore siblings of the same sex could not be identified on subsequent captures. After emergence from the pouch back-young were tagged in one ear with a numbered 1 cm fingerling tag (Saltlake City Stamp Co.).

3.2.3 Age estimation of sucklings

Data collected from known age field animals were statistically insufficient for the age estimation of ringtail possum sucklings in the field. Therefore the ages of wild young were estimated from growth data collected in captivity.

Studies on age estimation in marsupial young have used body measurements taken sequentially on animals of known age to develop an ageing key for animals in the wild of unknown age (Maynes, 1972; How, 1976). However, Wood *et al.* (1981) note that these studies do not appear to have taken into account between-animal variation. For example, fitted growth curves for individual animals may differ from each other although the curves may follow the same form. Between-animal variation will increase the width of the confidence intervals for the age of an animal, given a particular value for the body measurement (Wood *et al.*, 1981). In

addition, if sequential measurements, made on a number of individuals, are pooled and the data is treated as a single animal the fitted growth curve does not represent the true mean growth curve for all the animals measured, thus increasing error in the age estimation of an unknown age animal.

Growth data collected in this study from known age animals in captivity included measurements taken sequentially on the same animal. Therefore, growth curves were fitted to head length and pes length data collected for each individual ringtail possum suckling and an average growth curve was calculated for each body measurement from the mean of the coefficients. The ages of young caught in the field were estimated from the inverse form of the average growth curve.

This technique allowed for both between-animal and within-animal variations. Wood *et al.*, (1981) provide a method to calculate confidence intervals for the age estimation of an animal allowing for between-animal variation. However, this method requires extensive statistical and computational resources (Rose, 1989a). Such resources were not available in this study, hence those confidence intervals are not given.

The birth date of ten of the litters born in captivity was known to within ± 2 days. The median date was chosen as the day of birth for each of these litters. One litter was reared by a female (F1) who was in poor condition, as indicated by her low body mass and poor outward appearance (see Chapter 2). The young of this female appeared to grow abnormally slowly (young H and I, see Chapter 2). In addition, five sucklings (K, J, Q, R, and N) died prior to pouch emergence. Therefore, least squares regressions for age estimation were obtained using the growth data collected for the remaining ten young (seven litters) which were measured from birth to weaning.

3.2.4 Growth rates

Growth rate may vary over an individual's life and between individuals or species. Three different methods were used to estimate growth rate in the ringtail possum. The mean absolute growth rate for a given period was calculated by subtracting the mass (g) of an animal from the mass (g) at the following measurement and dividing by the difference in weeks.

The instantaneous relative growth rate (k) was derived from a plot of mass

(g) on a logarithmic scale, against age on an arithmetic scale (Maynes, 1976). The linear segments of such a plot were taken as the time when growth was occurring at a constant relative rate and 'k' ($\log_e \text{g.day}^{-1}$) was estimated from these segments as;

$$(\log_e W_2 - \log_e W_1) / (t_2 - t_1),$$

where W_1 and W_2 are mass of the young (g) at times t_1 and t_2 (days) (Huxley, 1932; Brody, 1945; Maynes, 1976). Instantaneous relative growth rates were estimated in this way for each individual animal from the time they released the teat. Individual values were calculated because of between animal variation in the length of time that 'k' remained constant.

Growth curves that have been used to describe mammalian growth include the logistic and Gompertz curves. Both these curves are sigmoid in shape. However, Lee and Cockburn (1985) suggest that the Gompertz curve more accurately describes marsupial growth because in contrast to the logistic curve it is asymmetrical and approaches an asymptote more gradually. The differential form of the Gompertz growth equation is;

$$G = -a \log_e S + b,$$

where G = specific growth rate; S = size of the organism; a = a constant that is directly proportional to the rate of growth and b = the specific growth rate when $\log_e S = 0$ (Kaufman, 1981). Lee and Cockburn (1985) fitted Gompertz equations to growth data from a range of marsupial species and obtained a value of ' a ' for each species.

Following the method of Kaufman (1981) individual Gompertz growth curves were fitted to the data collected for seven ringtail possum young reared in captivity for whom complete sigmoid growth curves were available. The growth data collected in this study was longitudinal (Kaufman, 1981). Therefore, the method involved initially estimating G as;

$$\frac{\log_e S_2 - \log_e S_1}{t_2 - t_1}$$

where, S_1 mass (g) at the beginning of the time interval (t_1) and S_2 is the mass (g) at the end of the time interval (t_2). Then G was plotted against the geometric mean of S_1 and S_2 (\bar{S}) on a logarithmic scale. \bar{S} was calculated as;

$$(S_1 \times S_2)^{0.5}.$$

Values for the constant proportional to the rate of decay of growth, i.e., ' a ', were obtained from the slope of the resultant straight line.

3.2.5 Development and Phases of lactation

The appearance of certain body characteristics (e.g., claws, vibrissae, pelage) and stages in the physical development of the young (e.g., release of teat, eyes opening, vocalisation, pouch vacation) were noted at each measurement of both captive and field young.

Information on the stages of growth and development of the sucklings enabled the period of lactation to be divided into the main Phases as defined by Tyndale-Biscoe and Janssens (1988).

3.2.6 Reproductive status

Tyndale-Biscoe (1955) showed a close correlation between testis weight and the presence of spermatozoa in the brushtail possum. Furthermore, Hughes *et al.*, (1965) notes that testicular regression in a large percentage of male ringtail possums is accompanied by a cessation of spermatogenesis. Therefore, assuming testis weight is indicated by testis volume (Setchell, 1977), testis volume was used in this study as an index of mating potential in males. The testis was assumed to be an oblate spheroid and testis volume was estimated from the following equation (Abbott and Hearn, 1978);

$$TV = \frac{\pi \times W^2 \times L}{6}$$

where, L = length of right scrotum (cm) and W = width of right scrotum (cm) and TV = index of testis volume (cm³).

During a particular breeding season, primiparous females were defined as females known not to have previously reared a litter and multiparous females were defined as those females known to have previously reared a litter. The breeding history of the females was assessed from their life history (i.e., if they were first caught as suckling young). The physical appearance of the pouch and teats allowed females to be classified as (Sharman, 1962; Hird, 1975);

- 1) Nonparous or juvenile females in which the pouch consisted of a loose flap. The skin lining the pouch in these females was dry and the degree of invagination was approximately 1 cm. The two posterior teats were small buds (approx. 2 mm) and everted, whereas the two anterior teats were

inverted.

- 2) Parous females which had reared a litter but were not breeding and had a small undeveloped pouch with a loose entrance. The degree of invagination was approximately 1.5 cm and the skin lining the pouch was dry with red-brown scales. Pouch hairs were obvious and stained red-brown, particularly around the teats. The teats were as for nonparous females.
- 3) Reproductively mature females in oestrus. These females had a deep pouch (degree of invagination approximately 2 cm) with a constricted entrance. The pouch lining was moist with a red-brown oily secretion.
- 4) Mature animals suckling pouch young.
- 5) Mature animals without pouch young but lactating with large mammary glands and extended teats. The pouch entrance was fully relaxed.

Since the majority of the females caught at the beginning of the study (June 1986) were carrying pouch young it was not possible to assess whether these females were primiparous or multiparous by the above method. Thus, the proportion of known primiparous females and multiparous females could only be determined for the 1987 and 1988 breeding season.

3.2.7 Population size

Estimates of population density were based on the number of animals (>700g) known to be alive (KTBA) and resident, that is, known to be present in the study area for at least two months (Pahl, 1985) i.e.,

$$KTBA = N_c + N_a,$$

Where N_c = total number of possums caught and N_a = number of animals present but not caught.

Population density estimates were only made for the Whitemark beach region of the study area because animals at this site were studied for longer and more intensively than animals in Paddies. Estimates were made for each month during which ringtail possums were caught, from June 1986 to June 1988.

3.2.8 Statistical methods

Differences between means were tested by two-tailed Student's t-tests or

analysis of variance (ANOVA; Sokal and Rohlf, 1969; BIOSSTAT). Chi-square analysis was used in the comparison of sex ratios (Caughley, 1977). Regression equations were calculated using the least squares linear regression method and are presented with the correlation coefficient (r) or the coefficient of determination (r^2). The 'significance' of the regression coefficient was tested using the standard method (Sokal and Rohlf, 1969). The 0.05 level of probability was accepted as indicating statistical significance.

3.3 Results

3.3.1 Pattern of growth

Values of head length, pes length, tail length and mass obtained for twenty of the animals reared in captivity are shown in Figure 3.2a,b and Figure 3.3a (the birth date of the young born in the wild was estimated from the equations obtained). The measured parameters increase with age, with the curves flattening out as the animal approaches maturity. Both pes length and tail length described a sigmoidal pattern of change with the steepest part of the curve occurring at the onset of emergence from the pouch. Head length showed a slightly different curve increasing in a linear fashion during pouch life. The mean head length of one day old ringtail possums was $5.3 \text{ mm} \pm 0.8$ ($n = 4$).

The mass of the young also showed a sigmoidal increase with increasing age similar to that shown by the pes length and tail length. However, the steepest part of the curve for this parameter occurred after the young had left the pouch and before it reached an asymptote after final weaning (W, Figure 3.3a). The mass of the three young measured from birth to maturity continued to increase after final weaning until they reached sexual maturity. Female A mated when she was 13 months old and weighed 890 g. Female S was not placed with a male and her mass continued to increase throughout her first year after reaching maturity. Male T mated when he was 16 months old and weighed 910 g.

Body measurements obtained for the litter reared by female F1 showed a slower increase with age after they left the pouch when compared with those of healthy young (Figure 3.3a).

An index of condition (body mass/ head length; Hocking, 1981) was

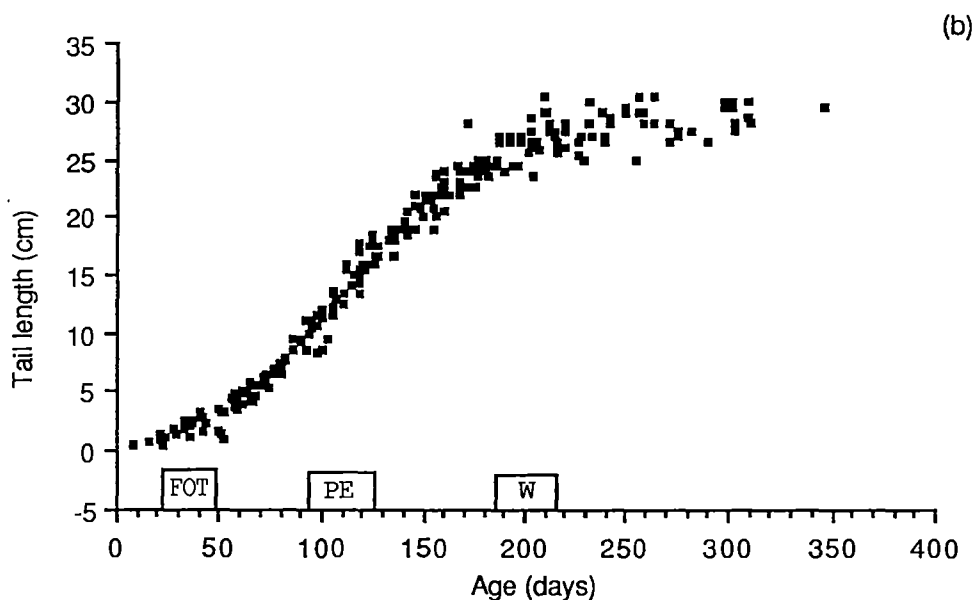
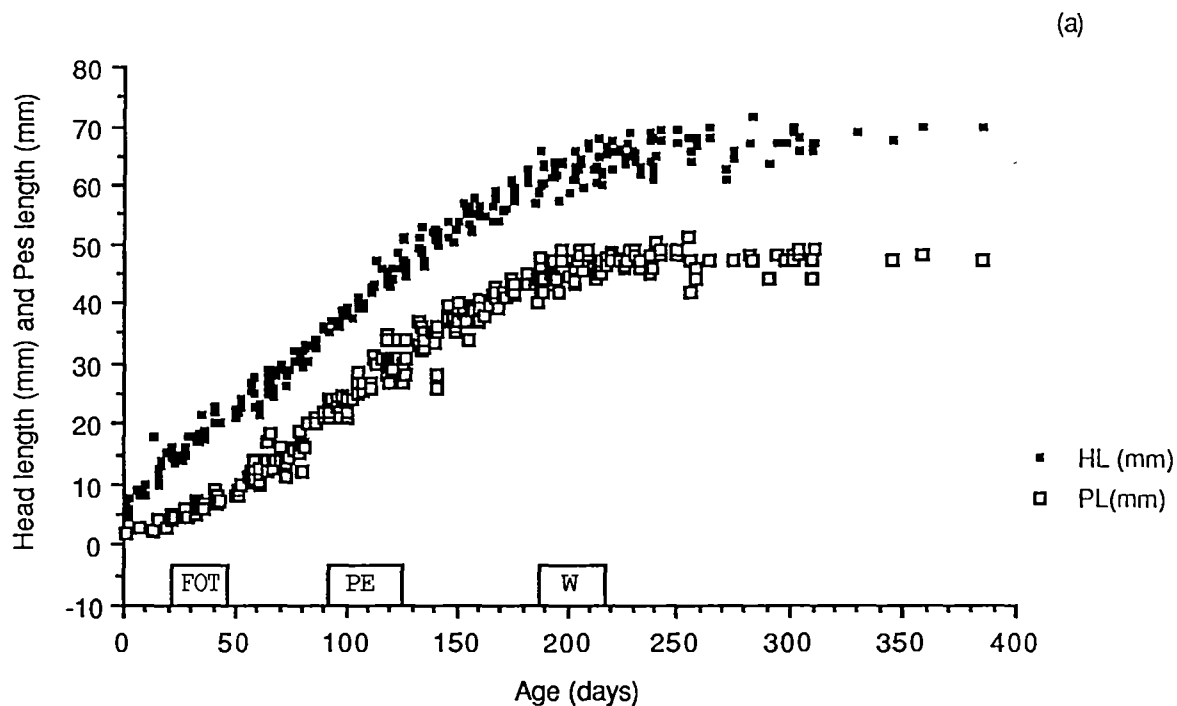


Figure 3.2 Growth of the head, pes and tail of the common ringtail possum (in captivity) from birth to maturity. Scatter of individual values from 20 young (11 litters).
FOT = Free of teat,
PE = Pouch emergence,
W = Final weaning.

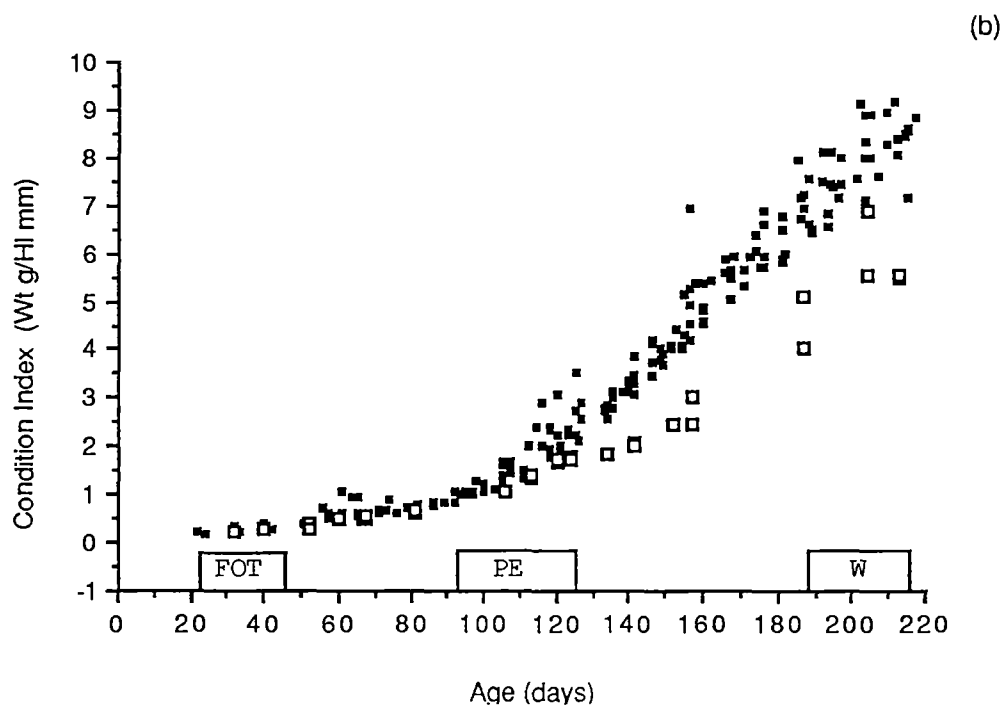
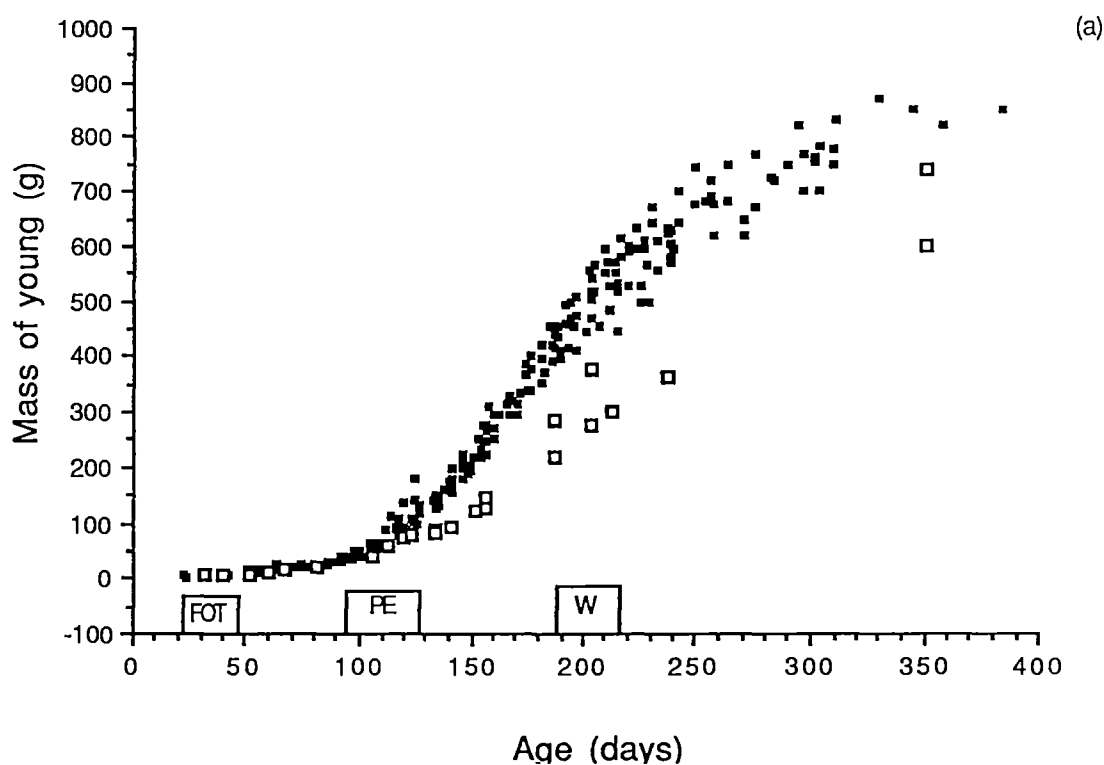


Figure 3.3 Change in (a) mass and (b) condition of ringtail possums with age. Scatter of individual values from twenty young (11 litters are given). Open squares are values for the two young reared by female F1 (see text). FOT = free of teat; PE = Pouch emergence; W = final weaning.

estimated for each young and plotted against age (Figure 3.3b). This relationship varied little compared with the results obtained using only body mass. However, it suggests that the rate of body mass gain by the young whose mother was in poor 'condition' was effected more than their skeletal growth.

These plots of measurements made on individual animals show that the degree of scatter increases after the young have left the pouch and as they approach maturity (Figure 3.2a,b and Figure 3.3a). This is due to the increase in between animal variation as the young grow and suggests that the accuracy of age estimation using these measured body parameters will decrease after the young have left the pouch.

3.3.2 Development and Phases of lactation

Table 3.1 summarises the timing of the major events which occur during the growth of the ringtail suckling. The young weigh approximately 0.3g at birth (Thompson and Owen, 1964) and subsequently become attached to a teat. Figure 3.4a shows a 17 day old young in the pouch.

At 36 ± 7 days (4-5 weeks) post-partum young were first recorded free of the teat (FOT). They remained in the pouch until they begin to emerge at 107 ± 7 days (about 15 weeks) post-partum (Table 3.1). Young returned to the pouch after it was first vacated but were recorded permanently out of the pouch (PE) at 116 ± 5 days (about 18 weeks) post-partum (Table 3.1, see also Figure 3.4c).

The young are finally weaned at 205 ± 7 days (about 27-30 weeks) post-partum. Janssens and Ternouth (1987), in comparing the composition of marsupial milk, defined weaning as the process which lasts from the time the young first ingest significant amounts of food other than milk, until the time when milk is no longer essential for the survival of the young. In this study because the time when the young first ingest significant amounts of solid food was not accurately determined the weaning period has been interpreted as the time when the young first has the opportunity to eat solid food (i.e. after pouch emergence) to when it has completely ceased sucking milk. Cessation of lactation has been called final weaning (denoted as W in the figures) and was estimated from whether milk was still secreted and/or observations on whether or not the young were still sucking.

Some body measurements of young at each of the above events are given in

Figure 3.4 Young ringtail possums at different stages of growth and development;

- (a) 17 day old suckling in pouch,
- (b) 77 day old (11 week) young in pouch,
- (c) 116 day old young just out of the pouch,
- (d) 135 day old 'back-young',
- (e) 168 day old 'back-young'.

(a)



(b)



(c)



(d)



(e)



Table 3.1 Stages in the development of young captive ringtail possums.

	n	mean \pm s.d (days)	Range (days)
Free of teat	8	36 \pm 7	22-46
First seen out of pouch	6	107 \pm 7	93-113
Permanently out of pouch	9	116 \pm 5	108-125
Duration of lactation or Final Weaning .	8	205 \pm 7	188-215

Table 3.2. The large range of values for each parameter at each event illustrates the variation in the timing of each event between individual litters. In general, there was no significant difference in headlength or mass between the sexes at each event in the growth of the suckling. However, the mean headlength of the male young at final weaning was significantly greater than that of the females (see Table 3.3).

The time of appearance of several observed external morphological characters showed little difference between individual young. Table 3.4 summarises the development of function and external characteristics in one individual female possum (see also Figure 3.4). One event which deserves special note is the onset of caecotrophy. The earliest observation made of a young performing caecotrophy was at 152 days after birth or 5 weeks after pouch emergence. Young were first observed eating leaves between 20 and 22 weeks post partum.

Events in the growth and development of the suckling ringtail can be used to divide the period of lactation into two main Phases as defined by Tyndale-Biscoe and Janssens (1988):

Phase 2 of lactation begins at birth and ends when the ringtail possum young begin to take food other than milk, (i.e., final pouch emergence). This Phase is divided into Phase 2a which covers the period of attachment to a teat and Phase 2b which begins when the teat is released.

Phase 3 of lactation covers the period from final pouch emergence to the cessation of lactation. During this Phase the ringtail possum young are either transported on the mothers back (hence 'back-young', see Fig 3.4e) or left in the nest. The young suckle intermittently, during this Phase, becoming less dependent on milk and more dependent on a solid diet.

These Phases of lactation and the corresponding events in the growth of the young are summarised in Figure 3.5. Hereafter, these particular stages of lactation will be referred to by the appropriate Phase.

3.3.3 Growth and life history of young in the wild

Figure 3.6a and 3.6b illustrate the mean mass and head length of young caught in the wild. The curves describe the average measurements obtained for young known to have been born during late autumn/early winter (i.e., the main birth season, see later). There were no significant differences in head length or mass

Table 3.2 Body measurements at particular stages of development in captive ringtail possums. Values are means \pm sd. n is also given. The range of values are in parenthesis.

Stage of development	Head length (mm)	Pes length (mm)	Tail length (cm)	Mass (g)
Free of Teat (FOT)	20.7 \pm 3.8 n = 17 (15 - 28)	8.4 \pm 2.29 n = 18 (4 - 11.1)	2.9 \pm 0.95 n = 15 (1.7 - 4.7)	7.8 \pm 3.57 n = 12 (3.7 - 13.4)
Permanently out of the pouch (PPE)	43.4 \pm 2.6 n = 10 (39.3 - 47.2)	26.7 \pm 3.57 n = 11 (21 - 31)	15.2 \pm 0.81 n = 13 (14 - 16.5)	97.4 \pm 19.92 n = 11 (78 - 135)
Final Weaning (W)	62.2 \pm 2.55 n = 11 (57.5 - 67.7)	45.3 \pm 2.0 n = 11 (42 - 49)	24.6 \pm 5.3 n = 15 (22 - 29)	473.5 \pm 51.5 n = 13 (362 - 541)

Table 3.3 Comparison of head length (mm) and mass (g) of male and female ringtail possums at various stages of growth. Values are given as mean \pm s.d with n in parenthesis.

Stage	Parameter	Female	Male	t-value	P
Free of teat	Head length (mm)	17.5 \pm 2.7 (22)	17.4 \pm 2.7 (12)	0.696	>0.05
Permanent pouch vacation	Head length (mm)	44.0 \pm 5.1 (12)	45.0 \pm 2.6 (10)	0.632	>0.05
	Mass (g)	94.7 \pm 29.6 (13)	100.3 \pm 25.0 (10)	-0.082	>0.05
Final weaning	Head length (mm)	61.7 \pm 2.5 (17)	63.3 \pm 1.4 (17)	2.194	<0.05
	Mass (g)	474.2 \pm 59.3 (17)	501.0 \pm 47.4 (17)	1.038	>0.05

TABLE 3.4 Description of developmental features from birth to maturity in a female ringtail possum.

Age (days)	Developmental features
3	Eye spots. Forelimbs developed and moving with a 'paddling' motion.
16	Pigmented, dark on dorsal surface; tip of the tail is unpigmented. Ear pinnae fused to the head and point forward. Young firmly attached to the teat (Fig 3.4a).
23	Eyes bulging and eye grooves just visible. Hind limbs formed with digits of the pes differentiating. Rhinarium similar to adult form.
52	Pouch visible. Mouth is open and young can release the teat. Ears still flat against the head but pointing backwards. Eye slits visible. Pigmented claws on manus and pes. Nasal and mystacial papillae.
67	Nasal and mystacial vibrissae present. Ear pinnae curling off head; lower incisors erupting; eyelids separating. On removal from the teat the young emitted a "chi-chi-chi-chi" vocalisation.
81	Orbital vibrissae present. Can grip with manus, pes and tail. Very vocal (Fig 3.4b).
107	Eyes open. Other vibrissae present. Fine hair covering body with thick ginger tuft at base of ear. Eyes are coloured olive. Young found out of pouch but returned when disturbed; can support own weight.
118	Out of pouch; very active; seen clinging to mothers back when she feeds at night. Mother responds to vocalisations made by the young. Black guard hairs nearly fully developed; tail white section has an orange tinge. Claws are still pigmented. Upper canines and incisors present (Fig 3.4c).
126	Complete pelage. Premolars through gum.
154	Eye coloration is now as adult, i.e., caramel. Claws white. Observed eating leaves alone. Emits a "chirupping" vocalisation when disturbed. White sections of pelage have lost their ginger tinge (Fig 3.4d and e).
189	Fully weaned. Caecotrophy observed during the day. Pouch deepening with dry red/brown secretion around teats. Posterior teats everted and anterior teats inverted.
338	Placed in cage with a single male.
368	Pouch deep (approx. 2cm) with constricted entrance. Red/brown moist secretion lining the walls of the pouch and around the entrance.
384	Gave birth to two pouch young.

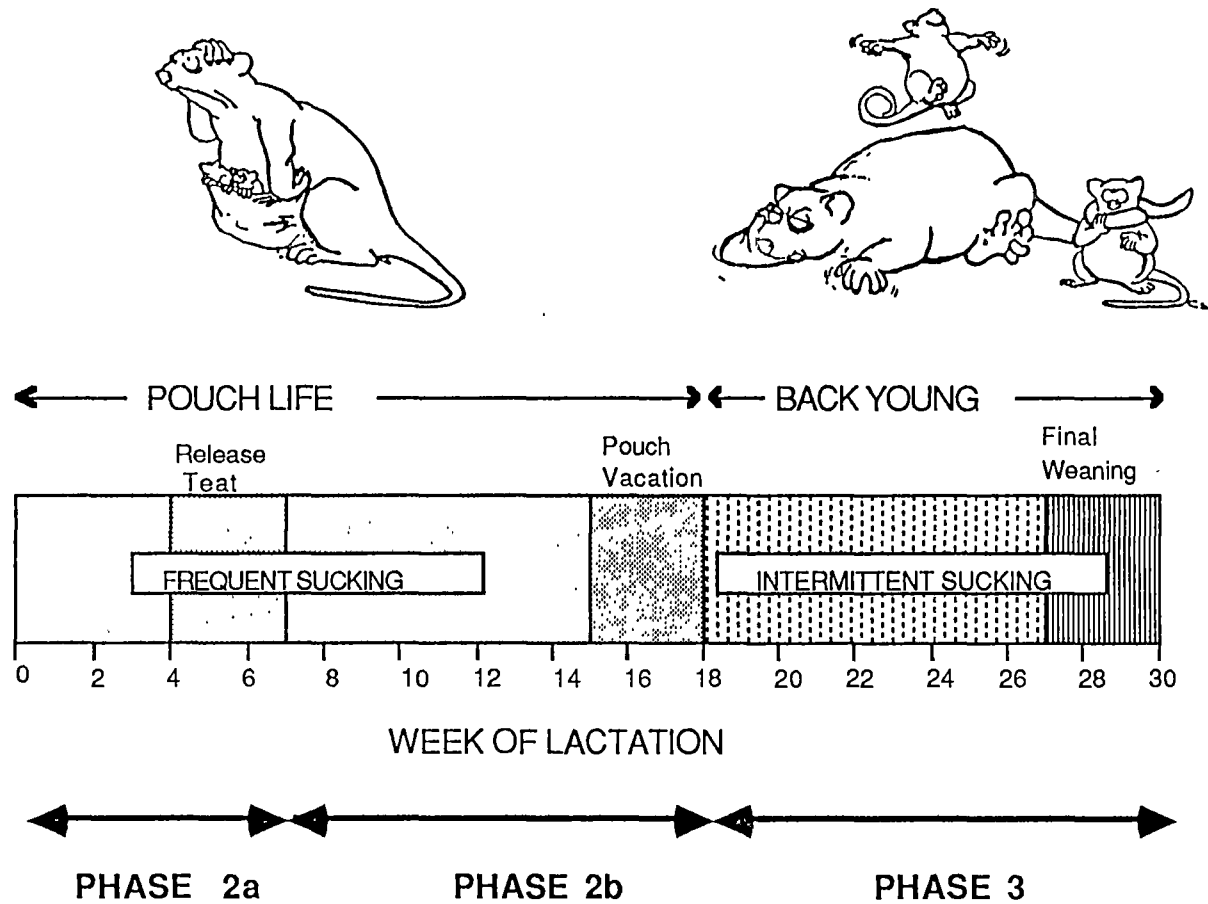


Figure 3.5 Summary of events in the growth of the ringtail possum suckling and corresponding changes in the Phases of lactation.

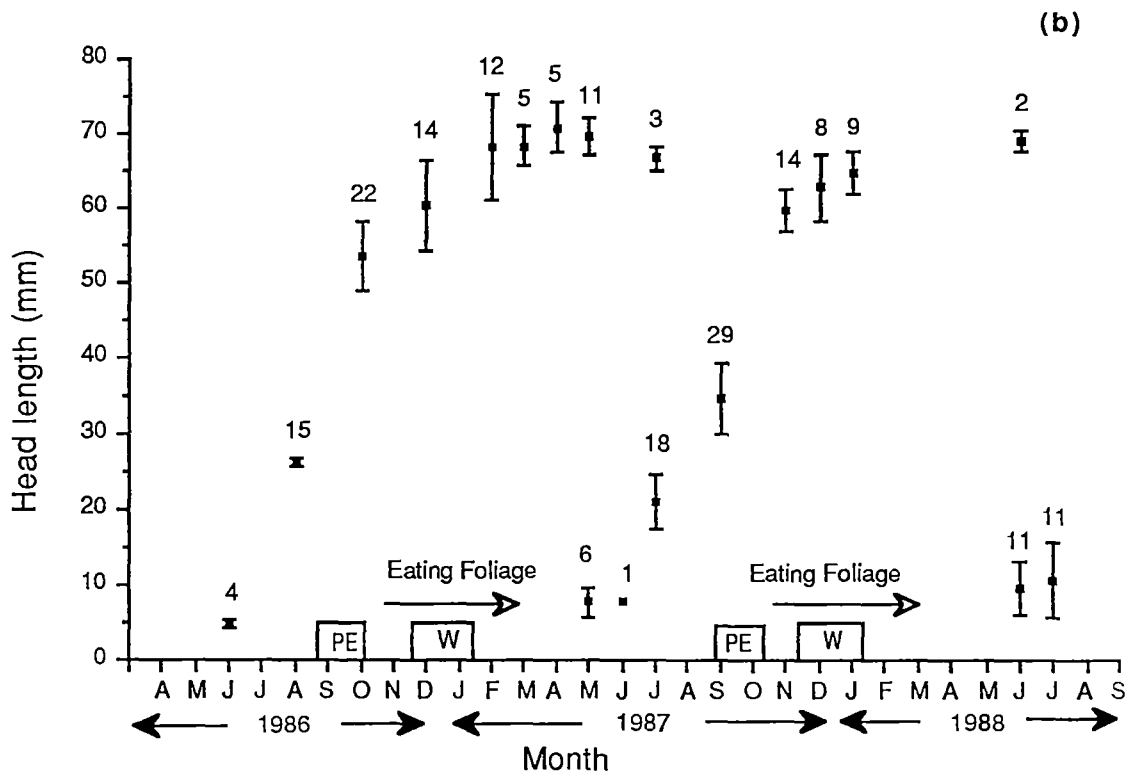
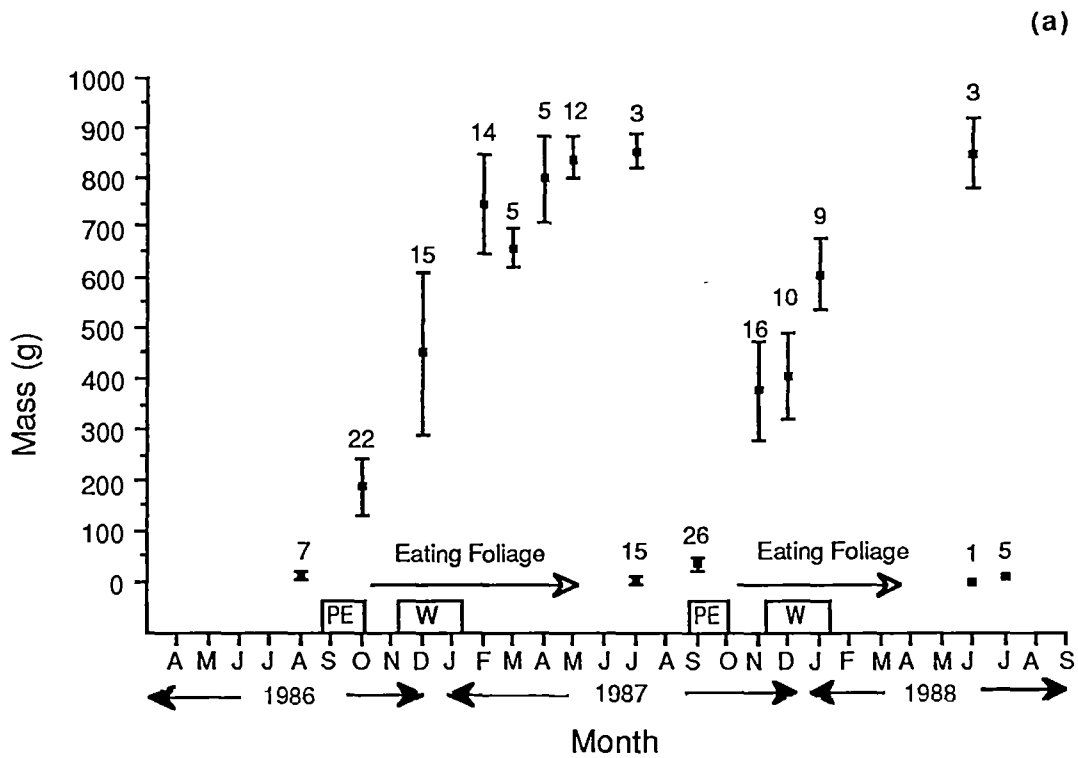


Figure 3.6 (a) mass and (b) headlength of wild ringtail possums known to have been born during late autumn/early winter. Values are means \pm SD. N is presented above bars. PE = pouch emergence, W = final weaning.

between the sexes during each of the months studied (unpaired t-test's, $P > 0.05$), so values obtained for males and females were combined.

The timing of various events in the life history of young in the wild were estimated from observations made at the time of capture and the growth and development observations of animals reared in captivity. For example, young were first observed eating leaves in captivity when they had a headlength of about 49 mm and they weighed about 170 g. Hence, it is assumed that young ringtail possums born in the wild during the autumn/winter months begin eating leaves during October (Figure 3.6).

Data collected on the mass and head length of a small number of young born during the spring and summer months are shown in Figure 3.7a and 3.7b. Young born during these months emerge from the pouch and begin eating leaves during May/June (Figure 3.7a,b).

3.3.4 Growth rates

The mean absolute growth rate (g/week) of the young up to weaning was variable (Figure 3.8). The trend was toward an increase in absolute growth rate up to week 23 of age. Paired t-tests conducted on a priori grounds showed no significant increase in absolute growth rate after week 23 ($P > 0.05$).

Table 3.5 gives the mean instantaneous relative growth rates ('k') for the nine ringtail young reared from birth to weaning. The stages A, B, C and D correspond to arbitrarily defined periods at which 'k' appeared to be relatively constant. Figure 3.9 illustrates these periods for one individual young. Each growth curve plotted for the eight other animals showed a similar pattern. The range of days over which 'k' appeared to remain constant in the nine young is given in Table 3.5.

During pouch life 'k' declines rapidly until it becomes constant at about 3.22% per day from 59 to 134 days (A, Table 3.5). This period covers the time at which the young emerge from the pouch (see Figure 3.9). The instantaneous

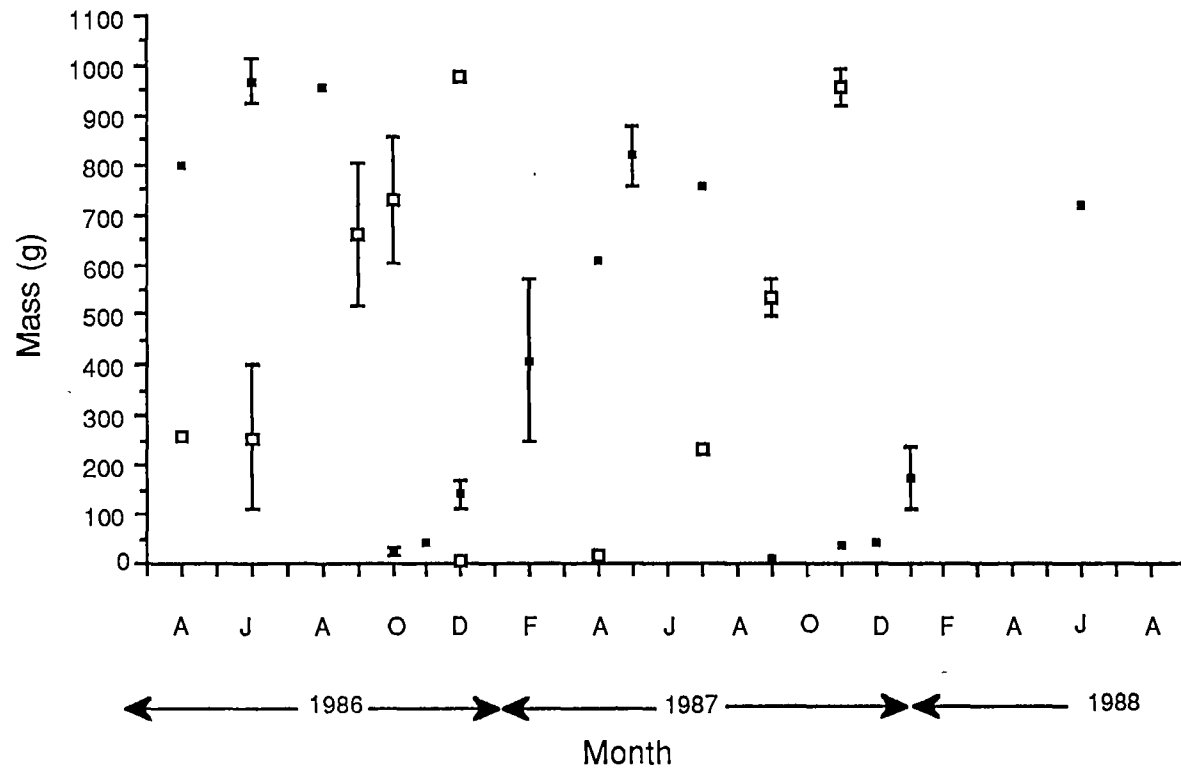


Figure 3.7 Mass of wild ringtail possum young known to have been born during the winter (■) and spring/summer (□) months. Values are means \pm SD.

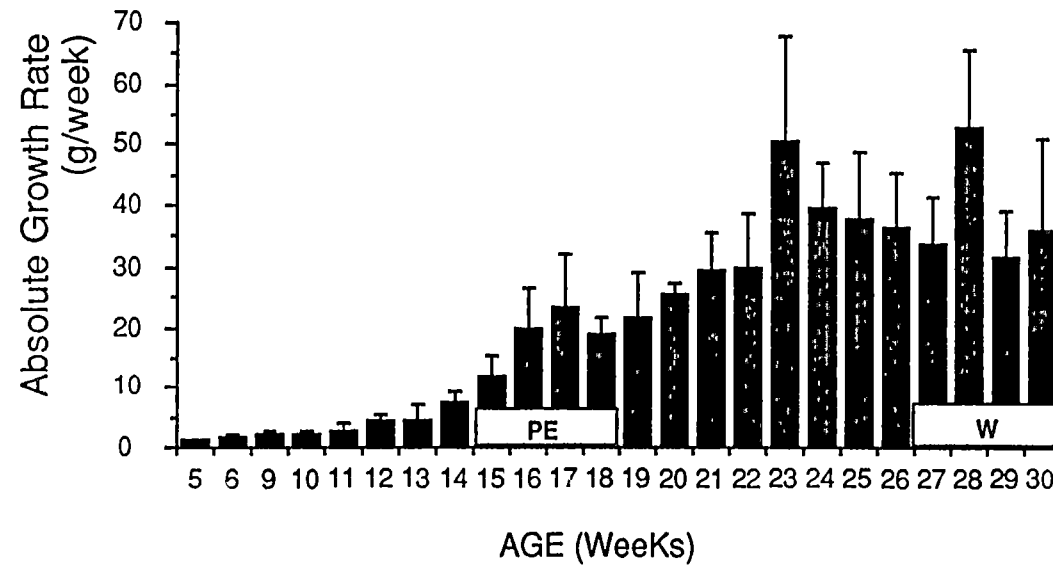


Figure 3.8 Absolute growth rate in ringtail possums from birth to weaning. Values are means \pm SD. PE = Pouch emergence. W = Final weaning.

Table 3.5 Instantaneous growth rate (k) in the ringtail possum.
Values given are means \pm s.d. Range and n in parenthesis.

Stage	Age Interval (days)	k value $\times 100$
A	59 - 134	3.22 ± 0.39 (2.42 - 3.85, $n = 9$)
B	123 - 188	2.27 ± 0.45 (1.61 - 2.85, $n = 9$)
C	171 - 233	1.94 ± 2.28 (0.73 - 1.45, $n = 9$)
D	211 - 384	0.38 ± 0.08 (0.24 - 0.46, $n = 9$)

relative growth rate further declines becoming constant again (i.e., $k = 2.27\%$ per day, Table 3.5) after the young have left the pouch (B, Figure 3.9). Another reduction in growth rate reduces 'k' to 1.94% per day over the period when the young are finally weaned (C, Table 3.5, Figure 3.9). After the young cease sucking milk, 'k' remains constant at about 0.38% per day (D, Table 3.5, Figure 3.9).

The Gompertz plot fitted to the growth data collected for one individual ringtail possum is given in Figure 3.10. The regression equations obtained for the seven individual ringtail possums for whom the most complete set of growth data was obtained are given in Table 3.6. All of the regression lines were significant ($P < 0.05$) and the mean Gompertz constant or 'a' (slope) was 0.01 ± 0.001 ($n=7$) (range $0.008 - 0.012$).

Most of the mass data for individual animals were collected using a time interval of 7 days (range 5 - 9 days). A correction factor for the Gompertz curve was estimated from the product of the slope and the largest time interval. Using the graph presented in Kaufman (1981) the approximate correction factor was found to be close to one. Hence the error involved in the estimation of 'a' was considered insignificant.

3.3.5 Age estimation and comparison of wild and captive growth.

Regression equations fitted to the growth data of individual sucklings were found to have a different form during and after pouch life. The growth curves derived from head length measurements were linear during pouch life. However, a quadratic term improved the description of the data (based on the coefficient of determination, r^2) after the young had emerged from the pouch (Table 3.7 and Table 3.8).

The animals measured were assumed to represent a random sample of the population. Measurements were taken regularly so the estimated curves were considered precise. There was no evidence that variability increased significantly with age from birth to final weaning, therefore assumption of normality on the original scale of measurement appeared reasonable. The mean growth equations obtained for pouch life and from pouch vacation to final weaning are given in Table 3.7 and Table 3.8, respectively.

A simple indication of whether the male and female ringtail young are growing in the same way may be obtained by comparing the coefficients of the growth curves obtained for each sex. There was no significant difference between

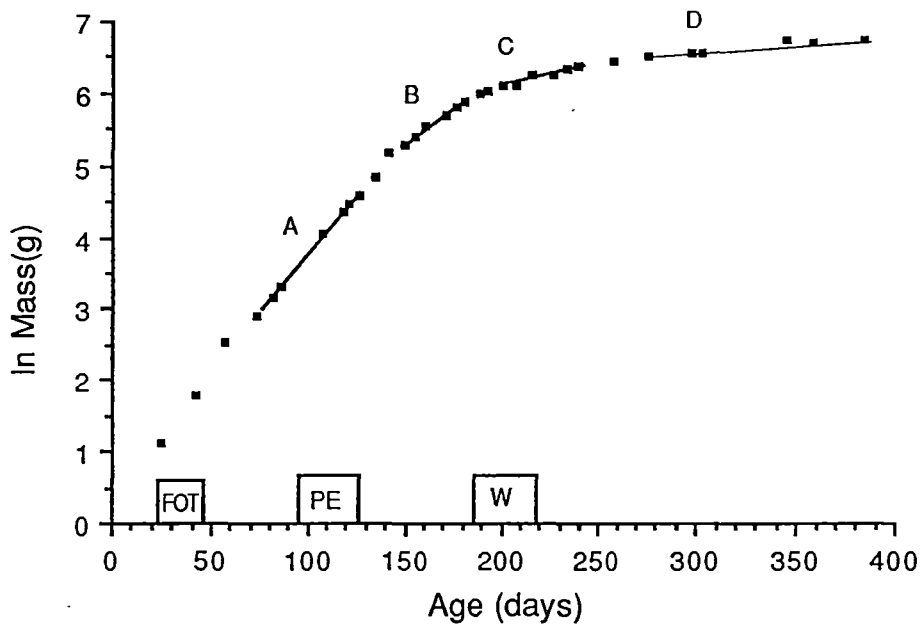


Figure 3.9 Periods of constant instantaneous relative growth (k) in a common ringtail possum (Young B).
 FOT = Free of teat
 PE = Pouch emergence
 W = Final weaning.

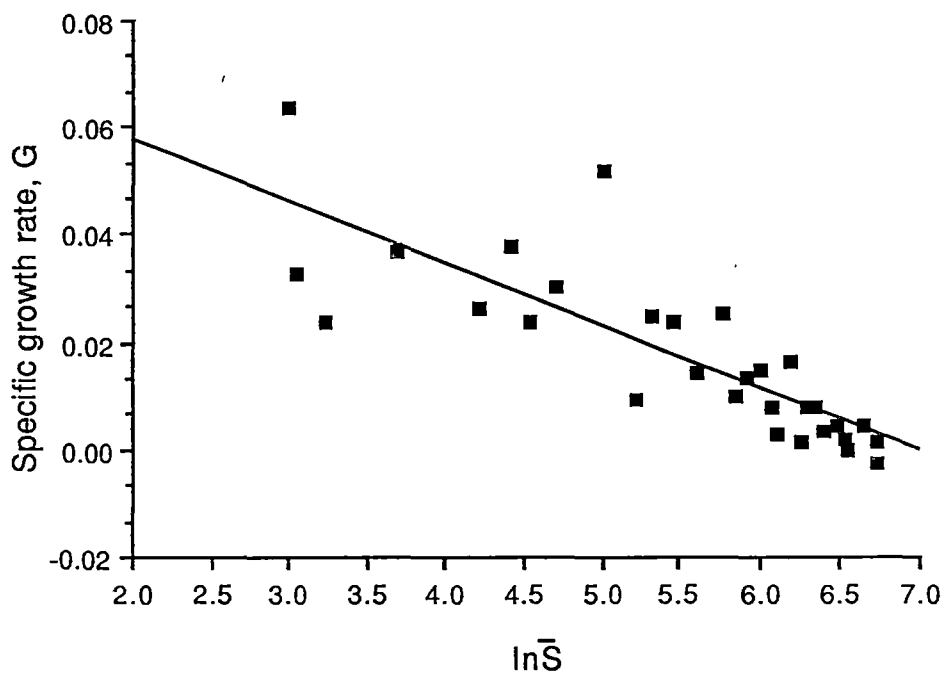


Figure 3.10 Gompertz plot fitted to the growth data of a ringtail possum (young B).
 \bar{S} = Geometric mean of the mass of the young at the beginning and end of a time interval (see text).
 $G = 0.081 - 0.012 \ln \bar{S}$, $r^2 = 0.675$

Table 3.6 Gompertz plots for individual ringtail possum young in captivity.

Young	Regression	t-value	P
A	$G = -0.009\bar{S} + 0.066$ ($r^2 = 0.457$, $n = 27$)	4.583	<0.01
B	$G = -0.012\bar{S} + 0.081$ ($r^2 = 0.675$, $n = 30$)	7.626	<0.01
C	$G = -0.011\bar{S} + 0.08$ ($r^2 = 0.701$, $n = 23$)	7.02	<0.01
D	$G = -0.009\bar{S} + 0.069$ ($r^2 = 0.272$, $n = 24$)	2.866	<0.01
E	$G = -0.008\bar{S} + 0.059$ ($r^2 = 0.516$, $n = 24$)	4.844	<0.01
F	$G = -0.011\bar{S} + 0.08$ ($r^2 = 0.685$, $n = 26$)	7.222	<0.01
G	$G = -0.01\bar{S} + 0.075$ ($r^2 = 0.509$, $n = 24$)	4.771	<0.01

Table 3.7 Regression lines describing growth in individual pouch young. y = head length (mm) or pes length (mm), and x = age (days).

Young	Head length	Pes length
A	y = 6.8206 + 0.309x (r = 1.00)	y = 2.4519+0.0422x+0.0017x ² (r = 0.99)
B	y = 7.2648+0.298x (r = 0.99)	y = 4.3216-0.0103+0.002x ² (r = 0.97)
C	y = 9.3528+0.2655x (r = 0.94)	y = 1.2084+0.0899x+0.0012x ² (r = 0.99)
D	y = 1.5778+0.3584x (r = 0.99)	y = 4.2881+0.2218x+0.0004x ² (r = 1.00)
E	y = 7.6403+0.3056x (r = 0.99)	y=-2.1624+0.2398x+0.00019x ² (r = 0.99)
G	y = 7.4529+0.3048x (r = 0.99)	y = 2.5508+0.052x+0.0018x ² (r = 1.00)
L	y = 4.8352+0.3452 (r = 1.00)	y = 3.2173+0.00019x+0.002x ² (r = 1.00)
M	y = 5.9103+0.3161x (r = 0.99)	y = 1.7962+0.1066x+0.001x ² (r = 0.93)
O	y = 6.2794+0.3196x (r = 1.00)	y = 1.6105+0.0718x+0.0014x ² (r = 1.00)
P	y = 7.8745+0.3137x (r = 1.00)	y = -0.3016+0.6123x+0.0009x ² (r = 1.00)
Mean		
Equation	y = 6.5009+0.3136x	y = 1.8981+0.1356x+0.0013x ²

Table 3.8 Regression lines describing growth in individual ringtail possums after emergence from the pouch until final weaning. y=head length(mm) or pes length(mm) and x = age(days).

Young	Head length	Pes length
A	$y = -29.7887+0.8395x-0.0019x^2$ (r = 0.98)	$y = -20.0617+0.5248x-0.0009x^2$ (r = 0.94)
B	$y = -28.2617+0.8171x-0.0019x^2$ (r = 0.98)	$y = -18.8353+0.4880x-0.0009x^2$ (r = 0.99)
C	$y = -17.5628+0.6431x-0.0012x^2$ (r = 0.98)	$y = -2.8072+0.279x-0.0001x^2$ (r = 0.97)
D	$y = 6.7836+0.3493x-0.0003x^2$ (r = 0.99)	$y = 0.9127+0.2413-0.00005x^2$ (r = 0.92)
E	$y = -71.0082-1.2846x-0.0031x^2$ (r = 0.98)	$y = 3.5932+0.2733x-0.0003x^2$ (r = 0.97)
G	$y = -21.6872+0.7957x-0.0019x^2$ (r = 0.98)	$y = -9.7446+0.477x-0.001x^2$ (r = 0.94)
L	$y = 10.9722+0.3477x-0.0005x^2$ (r = 0.99)	$y = -23.744+0.6304x-0.0014x^2$ (r = 0.95)
M	$y = 8.3392+0.422x-0.0007x^2$ (r = 0.94)	$y = 5.0185+0.2754x-0.0004x^2$ (r = 0.94)
P	$y = -6.7051+0.6132x-0.0015x^2$ (r = 0.97)	$y = -19.6144+0.5937x-0.0014x^2$ (r = 0.99)
Mean equation	$y = -16.5465+0.6791x-0.0014x^2$	$y = -9.4686+0.4313x-0.0006x^2$

the coefficients of the linear head length growth curve or the quadratic pes length curve obtained during pouch life for the male possums and the female possums (see Table 3.9). Similarly, there was no significant difference between the coefficients (a,b and c) of the quadratic curves obtained after pouch emergence for the male and female possums (see Table 3.9). Since there was no distinction in the relationship between the two parameters (head length and pes length) and age, the growth rates of males and females were the same.

The use of the growth equations to estimate the age of unknown sucklings was tested using seven known age captive young and three field young whose date of birth was known to within ± 1 day. The relationship between the age estimated from the head length equations and the actual age of these young (Figure 3.11) is given by;

$$\text{Estimated age from head length (days)} = 5.4646 + 0.9827 \text{ actual age(days)}, \\ r^2=0.978.$$

Similarly, Figure 3.11 illustrates the relationship between the age estimated from pes length growth equations and the actual age of the young, giving the following equation;

$$\text{Estimated age from pes length (days)} = 1.4584 + 0.8515 \text{ actual age(days)}, \\ r^2=0.961.$$

The relationship derived using the pes length equations shows the greatest deviation from the line of equality (45° diagonal) (see Figure 3.11). Therefore, head length measurements were used to estimate the age of unknown field sucklings.

The ages of 26 unknown age pouch young caught in the wild were estimated at first capture using the average head length growth equation (Tables 3.7 and 3.8). At subsequent recaptures of the same young, the age was calculated as the actual number of days elapsed between first and final measurements plus the age of the young when first measured (extrapolated age). The corresponding age of the young based on growth in captivity was calculated as at first capture ('same-size captive age'). These pairs of age estimates for sucklings were then plotted against each other (Figure 3.12).

Any significant difference in the change in growth (i.e., growth rate) between young reared in the field and those reared in captivity should be indicated by a significant departure from the line of equality. However, the slope of the regression line obtained for the relationship between 'same-sized captive growth' and

Table 3.9 Comparison of male and female growth equations.

Stage	Measurement	Unpaired t-test					
		Coefficient a		Coefficient b		Coefficient c	
		t-value	P	t-value	P	t-value	P
Pouch life	Head length	0.496	0.63	-0.774	0.5		
	Pes length	-1.21	0.26	1.241	0.25	-0.315	0.76
Back young	Head length	0.524	0.62	-0.193	0.85	-0.113	0.91
	Pes length	1.33	0.22	-1.15	0.29	0.925	0.39

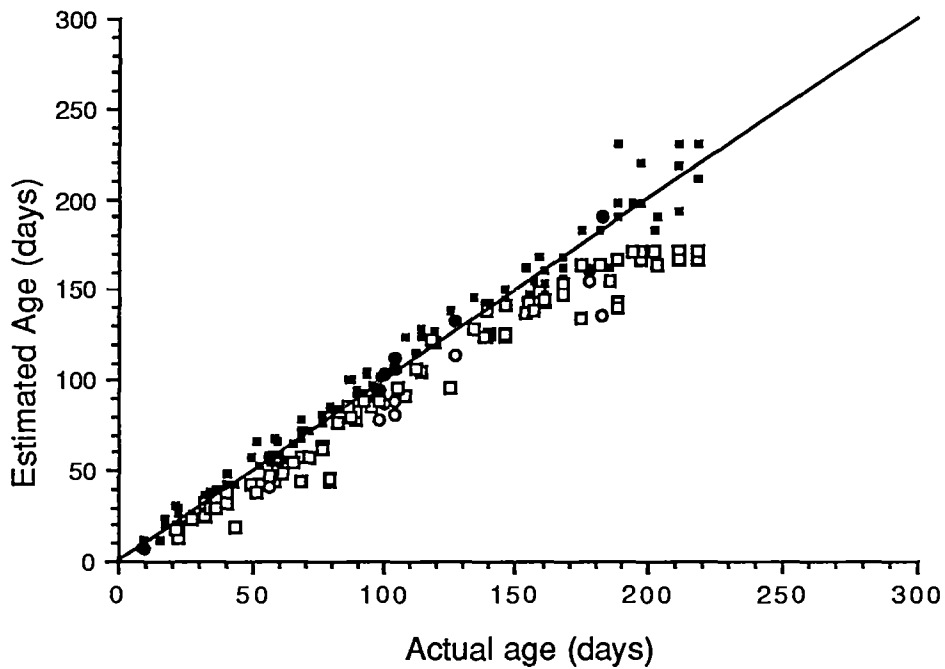


Figure 3.11 Relationship between age estimated from head length equations (■, ●) and pes length equations (□, ○), and the actual age of young ringtail possums.
 ■ □ = captive known age young (n = 7)
 ● ○ = known age wild young (n = 3)
 Line of equality ($y = x$) indicated.

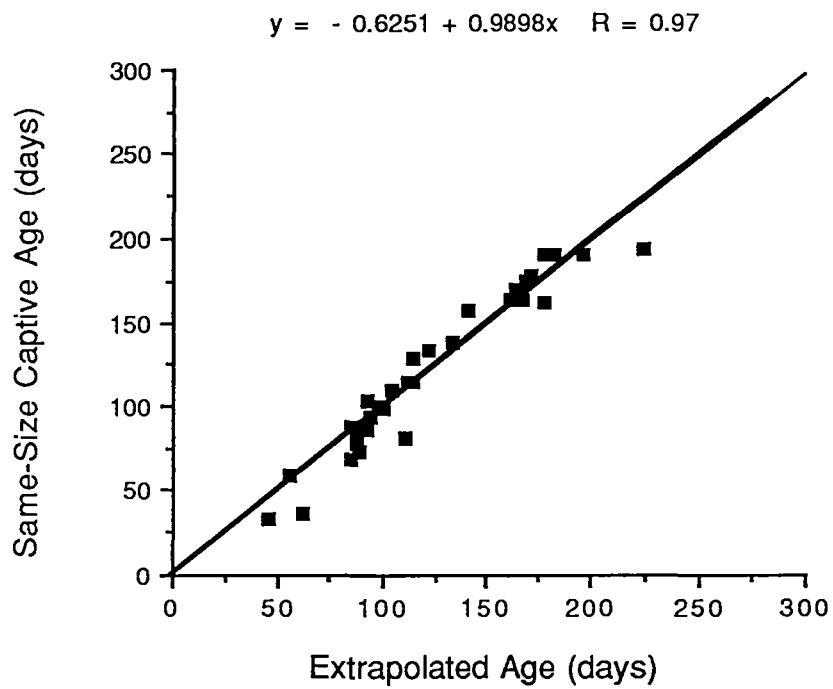


Figure 3.12 Relationship between age of wild young based on growth in captivity and age of wild young extrapolated from field captures. N = 38. Line of equality indicated (i.e., shorter line)

extrapolated growth showed no significant deviation from the line of equality (see Figure 3.12). Therefore, the rate of growth of field and captive sucklings appeared to be similar.

3.3.6 Breeding season

The majority of ringtail young in the study population were born between May and August. However, a small number of births were also recorded in all other months of the year, except for November and December (Figure 3.13). Gestation length estimated from observations of matings and births in captivity ranged from 20 to 26 days.

The date of birth for each unknown age pouch young was estimated from the head length growth equations (see earlier). The mean date of birth was calculated as June 22 for 1986 and June 18 for 1987. The mean date of birth of young born to primiparous females was June 21 (± 19 days, $n=13$) and the mean date of birth of young born to multiparous females was June 13 (± 21 days, $n=56$) when the data collected for 1987 and 1988 were combined.

Three groups of births throughout the year were recognised. Firstly the main autumn/winter birth season (i.e., May/June and July), secondly the slightly later births of young born to primiparous females (i.e., August/September and October) and finally the young born during the summer months (i.e., January and February) (Figures 3.6 and 3.7).

The births of young in captivity occurred during June, July and August. As in the wild population, primiparous females gave birth slightly later than multiparous females (see Table 3.10). One primiparous female (12.1 months old) which had been born in captivity gave birth to two young on July 26 1987, however, the young subsequently died five weeks later. Six weeks later this female mated again and gave birth to two young, however, these young also died six weeks later. Only one female, caught in the wild, that was known to have lost her young, subsequently reproduced for a second time.

Figure 3.14 illustrates the pattern of change in testis volume index for adult males throughout the months studied. The size of the testis as indicated by the testis volume index, was greatest during late summer/early autumn prior to the main birth season, and lowest during the spring and early summer months. Assuming this

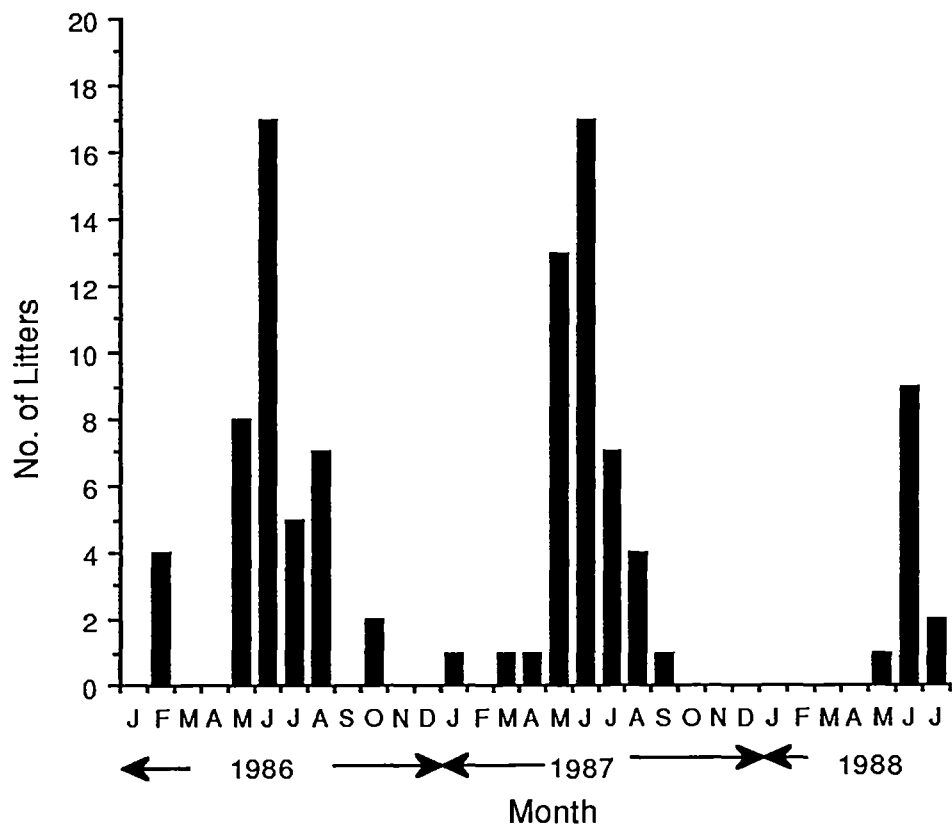


Figure 3.13 Seasonal distribution of births in wild ringtail possums.

Table 3.10 Details of births in captivity.

Mother	Mass of mother (g)	Multiparous (M) or Primiparous (P)	Litter size	Date of birth	Sex of young
F1	940	P	2	21 July	F
F3	870	P	2	5 July	M
F2	-	M	2	9 June	M,F
F4	950	P	1	6 Aug	M
F5	960	M	2	6 Aug	M,F
F1	930	M	2	14 June	F
F6	910	M	2	26 July	M,F
F5	1010	M	2	15 June	M,F
F4	950	M	2	16 June	F
F7	950	P	1	28 July	F
A	850	P	2	26 July	M
A	880	P	2	6 Oct	M,F

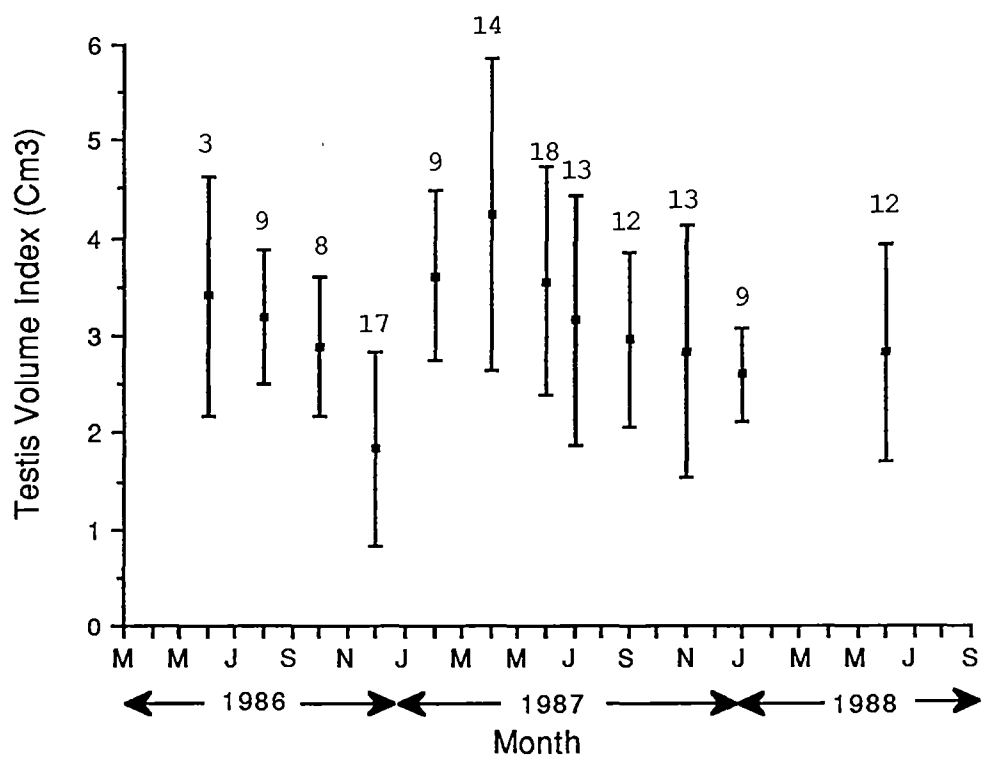


Figure 3.14 Testis volume index for wild ringtail possums. Values given are means \pm SD. N is given above error bars.

increase in testis size reflects the onset of spermatogenesis the main mating season was estimated to be between March and May. However, as indicated by the timing of births, some males may mate during other months of the year (Figure 3.13).

Figure 3.15 summarises the timing of reproductive events throughout the year for female ringtail possums reproducing during the main autumn/winter breeding season at Whitemark Beach and Paddies.

3.3.7 Litter size and sex ratio of sucklings

Females reared, on average, one or two young per year. There were no records in the field or in captivity of females suckling more than two young. The mean litter size in the field was 1.84 ± 0.37 ($n = 85$).

The mean litter size of females known to be primiparous was significantly smaller than that of females known to be multiparous ($df = 64$, $t = 2.16$, $P < 0.05$, Table 3.11). Similarly, the mean litter size of primiparous females in captivity was less than that of multiparous females (i.e., 1.7 ± 0.51 , $n = 5$ and 2.0 ± 0.00 , $n = 6$, respectively).

The sex of the young was determined for sixty nine litters observed in the wild. Sex ratio of these young were 51 males : 63 females or 0.55 when expressed as the proportion of females. However, a chi-square test showed that this difference was not significant ($P > 0.05$).

3.3.8 Frequency of litters and annual fecundity

Seven multiparous females which had reared a litter born during the autumn/winter, gave birth to a second litter in the spring or summer months. These births represented 12% of the total number of litters produced by multiparous females over the three breeding seasons. Three of these occasions were recorded during 1986, three during 1987 and one during 1988. No known primiparous females were double breeders.

The mean annual fecundity of the wild population did not vary significantly between 1986 and 1987 (Table 3.12). Annual fecundity for 1988 was not estimated because animals were only caught in February and June of this year. The overall mean annual fecundity for 1986 and 1987 was 1.91 offspring per female.

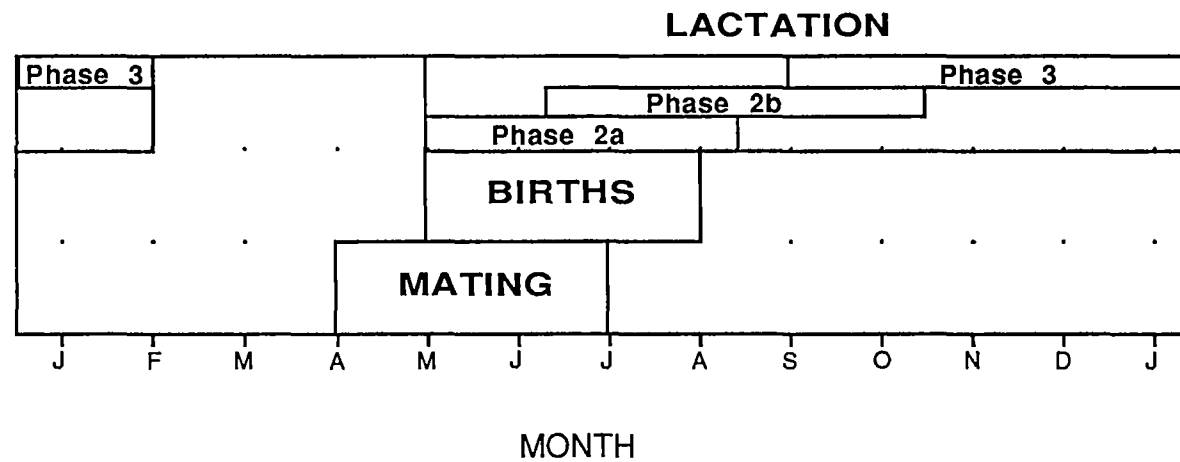


Figure 3.15 Timing of reproductive events in the ringtail possum.

Table 3.11 Mean size of litters produced by female ringtail possums during 1986, 1987 and 1988.

Female Group	Litter Size		n
	Mean	SD	
Primiparous	1.615	0.51	13
Multiparous	1.851	0.51	47

Table 3.12 Mean annual fecundity of the ringtail possum population at Whitemark Beach and Paddies.

Year	No. of females producing litters	No. of young produced	No. of offspring per female
1986	36	71	1.97
1987	35	65	1.86

3.3.9 Duration of lactation

The duration of lactation (estimated from the age of the young at final weaning) for wild females breeding once a year (single breeders) was similar to that found for animals in captivity (Table 3.13). However, data collected on three multiparous females (for whom the age of the young could be estimated) which reared a second litter (double breeders) suggest that these females weaned their first litter earlier than single breeders (see Table 3.13).

3.3.10 Adult body mass and condition

The body mass of both male and female adult ringtails varied seasonally (Figures 3.16). A 2-way ANOVA showed no significant difference between the mean body mass of the sexes ($F_{1,368} = 0.033$, $P > 0.05$). However, a significant difference was found in the pattern of variation in body mass shown by each sex throughout the year ($F_{11,368} = 3.585$, $P < 0.001$).

Males were heaviest in the summer and lost weight during the autumn mating season, reaching their lowest mass during the winter months (Figure 3.16a). The female's body mass was generally lowest during autumn and early winter (April/May/June) when the majority mated and gave birth to their young. Their mass then increased during the remaining winter months reaching a peak in late winter and early spring (August/September) before declining during the late spring and summer months (Figure 3.16b).

Figure 3.17 illustrates the mean change in mass of female ringtail possums during lactation. The body mass of females tended to increase while the young were suckled in the pouch. There was no significant difference between the mass increase of females during Phase 2a and females during Phase 2b of lactation. However, after the young have left the pouch (Phase 3 of lactation) the females generally lose weight.

The mean body mass of primiparous and multiparous females producing young during the autumn/winter measured within one month of the births was $895.6 \text{ g} \pm 88.9$ ($n=9$, range = 750-990) and $1037.7 \text{ g} \pm 96 \text{ g}$ ($n=37$, range 830-1240), respectively. This difference was significant ($t = 4.051$, $P < 0.05$), hence multiparous females weighed, on average, more than females producing young for the first time.

Table 3.13 Duration of lactation in wild ringtail possums.

Breeding frequency (per year)	Mean (weeks)	s.d	n
Single Breeders	30.5	± 3.1	13
Double Breeders	23.2	± 2.8	3

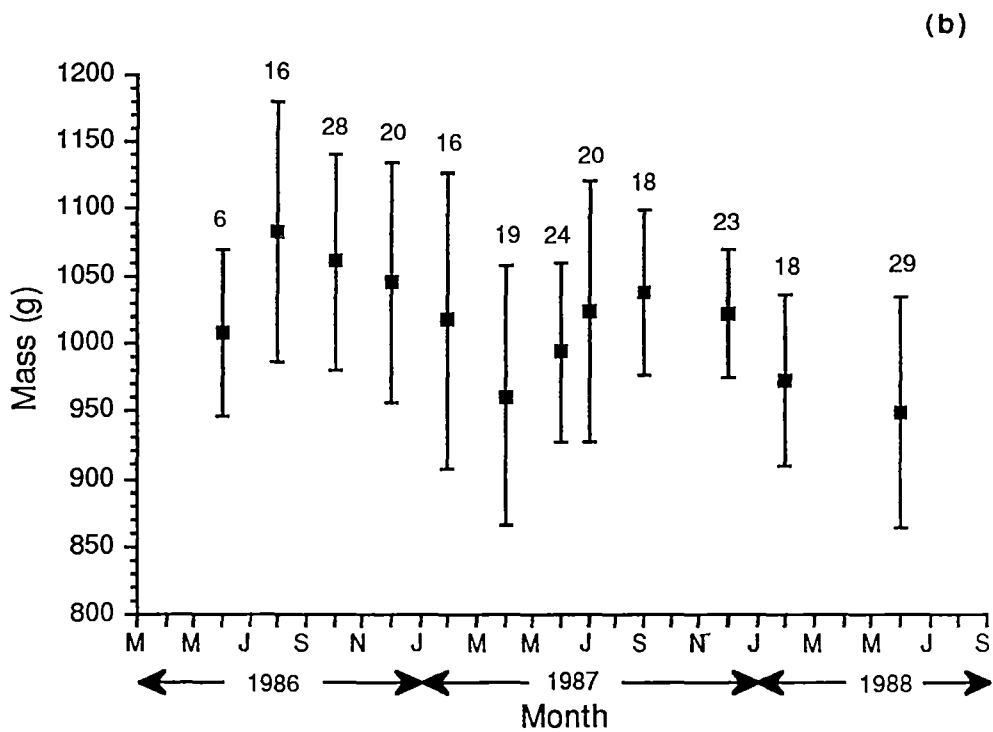
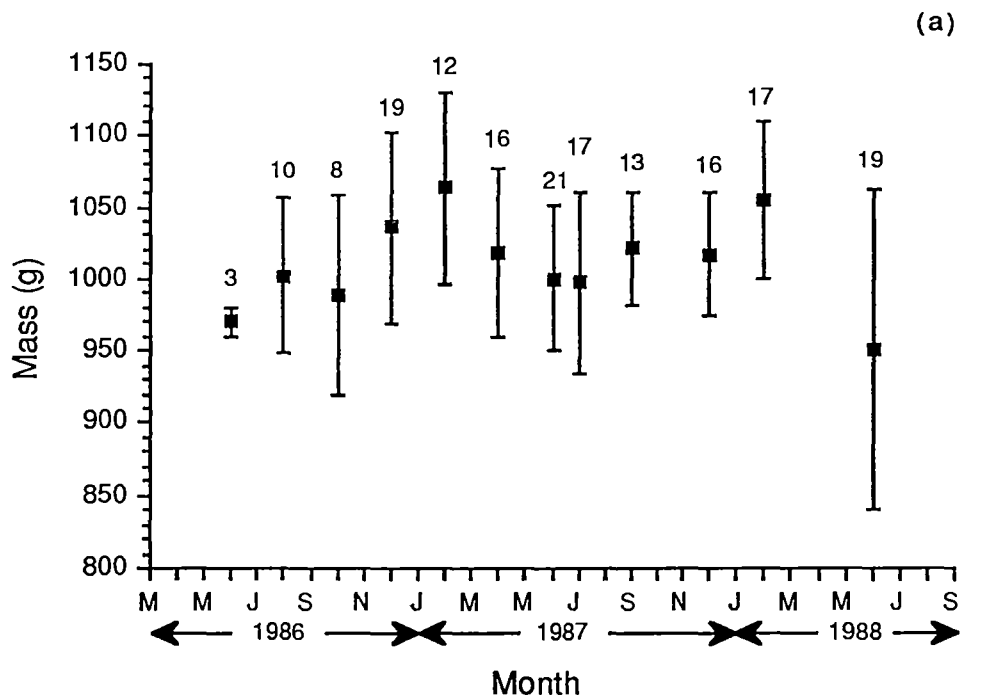


Figure 3.16 Seasonal changes in body mass in (a) adult male and (b) adult female ringtail possums. Values given are means \pm SD. N is given above error bars.

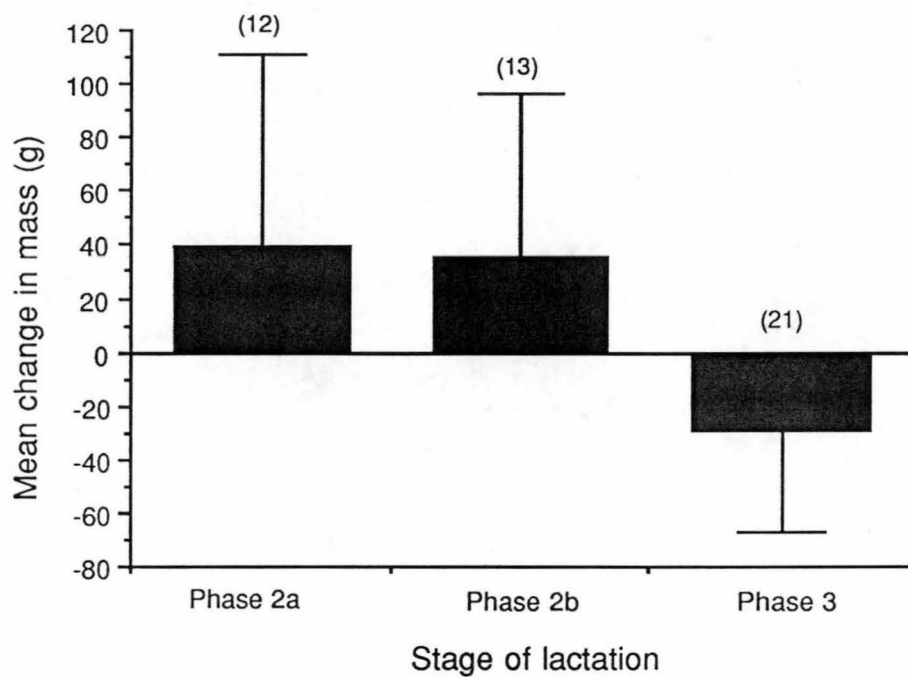


Figure 3.17 Change in mass of lactating ringtail possums. Values are means \pm SD. N is in parenthesis.

3.3.11 Population size and adult sex ratios

The population size estimates for the Whitemark beach region of the study area were low in June 1986 because of the initial discovery of dreys and animals. Estimates of the number of animals fluctuated between months but there was no obvious pattern in the variation (Table 3.14). The lower numbers during the summer and autumn months may have been due to emigration of sub-adult animals.

The highest average records for the number of individuals in the 16 hectare Whitemark Beach region of the study area were 38 during May/June/July and Nov/Dec 1987 (Table 3.14), giving an estimated density of 2.37 ha⁻¹. The lowest density recorded (25 individuals or 1.56 ha⁻¹) occurred during Jan/Feb 1988 (not including June 1986). The highest estimate for KTBA in the Paddies region of the study site was 37 animals during the March/April, May/June 1987 fieldtrips giving a density of 2.47 individuals.ha⁻¹.

Females outnumbered males in eight capture periods (Table 3.14). However, the difference was only significant in October 1986 ($\chi^2 = 4.57$ and , $P < 0.05$).

3.4 Discussion

3.4.1 Pattern of growth

The sigmoidal pattern of growth shown by the ringtail possum is similar to that described for a number of marsupial species e.g, *Trichosurus vulpecula* (Lyne and Verhagen, 1957), *Antechinus stuartii* (Marlow, 1961), *Macropus parma* (Maynes, 1976), *Cercartetus caudatus* (Atherton and Haffendon, 1982), *Thylogale billardierii* (Rose and McCartney, 1982). An exception to the general pattern of growth in ringtail possums was the growth of the head. During pouch life the head length increased linearly until its growth rate began to decline as it reached adult body size. Other studies have also noted a linear pattern of growth of the head in ringtail possum pouch young (Thompson and Owen, 1964; Hird, 1975). The tendency for head length to show the most rapid development during pouch life and the low age-specific variation in head length suggests a precedence for the growth of

Table 3.14 Number of ringtail possums (> 700g) known to be alive (KTBA) and the ratio of the sexes in the Whitemark beach region of the study area from June 1986 to June 1988.

Month	Males (KTBA)	Females (KTBA)	Sex ratio (proportion of females)	Chi-square test
June 1986	3	7	0.7	
August 1986	14	14	0.5	
October 1986	12	25	0.67	P<0.05
December 1986	12	16	0.57	P>0.05
February 1987	18	16	0.47	P>0.05
March/April 1987	15	16	0.51	P>0.05
May/June 1987	21	17	0.44	P>0.05
July 1987	17	21	0.55	P>0.05
September 1987	18	13	0.42	P>0.05
Nov/Dec 1987	20	18	0.47	P>0.05
Jan/Feb 1988	11	14	0.56	P>0.05
June/July 1988	15	22	0.59	P>0.05

the skull in pouch young.

Measures used to describe growth may be divided into two categories; (1) those which measure only positive growth and (2) those which measure both positive and negative growth (Hocking, 1981). The skeletal measurements used in this study (head length, pes length and tail length) are included in the first category. Body mass is an example of the second category since body mass of a growing young may decrease as a result of decreased food intake.

The greater influence of nutrition on body mass compared with the growth of the skeleton is possibly illustrated in this study by the pattern of growth obtained for the two sucklings whose mother was in poor condition. The body mass of these young increased at a slower rate after the onset of pouch emergence than that of young reared by healthy mothers. However, the differences in the rate of growth of the head, pes and tail of these young compared with young reared by healthy mothers were less pronounced. It seems reasonable to assume that the poor condition of the mother would result in lower milk production, however this will be further discussed in Chapter 8.

Skeletal measurements are generally considered the most reliable indicators of growth because they are not subject to variation in the fullness of the digestive tract or loss of body fluids (Klein, 1964). However, skeletal measurements may not provide the overall picture of growth of the body since closure of epiphysial cartilages prior to the completion of growth in body size may occur (Hocking, 1981). This is illustrated in the present study by the earlier age at which head, pes and tail length reach an asymptote when compared with body mass. By the time the young are fully weaned their head, pes and tail have all reached an asymptotic length. However, the overall body size of the ringtail possum as indicated by body mass continues to increase up to at least a year after it has reached maturity.

Body mass is also a more sensitive indicator of the short-term changes in body size. Hence it indicates short-term changes in the condition for growth. This is illustrated by the weekly mass increment of ringtail possum young or mean absolute growth rate which shows a general increase with age but also indicates periods of negative growth. Body mass is an essential measurement for the comparison of growth rates in animals of different body form (Russell, 1982).

3.4.2 Development

Ringtail possum young were first found to have released the teat at four weeks post-partum which is about two weeks earlier than previously reported (How *et al.*, 1984, Table 3.15). However, the time of permanent pouch vacation and the mass of *P. peregrinus viverrinus* at this time was similar to that reported for *P. peregrinus* in other studies (Table 3.15). Furthermore, the general development of external and functional features noted for the pouch young in this study was similar to the sequence outlined in detail by Thompson and Owen (1964) and the observations made by Presidente (1982).

The age at which a marsupial leaves the pouch is proportional to its mothers body mass (Russell, 1982). Ringtail possum young emerge from the pouch later than predicted from the allometric relationship of Russell (1982), which is based on a range of marsupial species. However, when pouch life is expressed as a proportion of maternal mass to the power 0.27 (Russell, 1982), they leave the pouch earlier than the young of other marsupial arboreal folivores e.g., *Trichosurus vulpecula* (Smith *et al.*, 1969), *Petauroides volans* (Russell, 1982), *Phascolarctos cinereus* (Smith, 1979a). A factor influencing the length of pouch-life may be the energy expenditure involved in carrying the young in the pouch (Russell, 1982). The ringtail possum has an average litter size of two whereas the other marsupial arboreal folivores rear a single young. Therefore, selection may have favoured a shorter pouch life in the ringtail possum to reduce the energy cost of carrying the two young. Physical constraints may also affect the length of pouch life, two rapidly growing young would take up more room in the pouch than one young. Janssens and Messer (1988) argue that a factor initiating pouch exit is the increasing thermal incompatibility of mother and young. This may occur at an earlier stage when two young are reared rather than one.

The age range at which the ringtail possum young were weaned in this study was similar to that reported in other studies (Table 3.15). The length of lactation in the ringtail possum is 129% of that predicted allometrically from other marsupial species and 141% of that predicted from data on other herbivores (Russell, 1982). This relatively long period of dependence on the mothers milk, is shared by other arboreal marsupial species e.g., *Petauroides volans* (Smith,

Table 3.15 Development in *Pseudocheirus peregrinus*.

Site	Adult female body mass (g)	First off teat (FOT) (d)	Wt at FOT (g)	Pouch exit (d)	Wt at PE (g)	Fully Weaned (months)	Wt at weaning (g)	Reference
Warramate (Victoria)	771	-	-	120	80-90	6-7	400	Thompson and Owen (1964)
Lysterfield (Victoria)	773	-	-	124	100	5-7	253	Pahl and Lee (1988)
Sandy Point (Victoria)	713	-	-	-	100	-	300	Hird (1975)
Sandy Point (Victoria)	770	42	-	125-130	65-90	6-7	-	How <i>et al.</i> , (1984)
Sandy Point (Victoria)	750.5	-	-	124	100	5-8	291	Pahl and Lee (1988)
Flinders Island (Tasmania)	1014	22-46	8	108-125	97	5-7	359* 473#	This study

* = field young, # = captive young

1969), *Dendrolagus matschiei* (Olds and Collins, 1972), *Trichosurus caninus* (How, 1976), *Phascolarctos cinereus* (Smith, 1979).

Cessation of lactation, either naturally or after the loss of pouch young, occurred over a seven day period which was the minimum time recorded between milk being secreted from the gland and failure to extrude milk. This is a similar time to that observed in *T.vulpecula* (Sharman, 1962; Cowan, 1989).

3.4.3 Growth rate

The relative growth rate of an animal usually decreases as the animal increases in size (Bertalanffy, 1960). However, this decrease in growth rate is not necessarily constant. Several studies of marsupial species have shown that decreases in growth rate are interrupted by periods of constant rates of growth (i.e., *Macropus parma* (Maynes 1976), *Bettongia gaimardi* (Rose 1989b), *Potorous tridactylus* (Bryant, 1989). Similarly, growth of the ringtail possum showed periods when 'k' was constant.

Growth in the ringtail possum may be divided into several stages according to the rate of growth. The first stage, from birth to just prior to pouch emergence, when the actual mass increment per week increases steadily, involves a rapid decline in the relative instantaneous growth rate ('k'). However, the highest 'k' values were estimated during this stage. This early stage of rapid growth has also been noted in other marsupial species e.g., *Macropus parma* (Maynes, 1976), *Trichosurus vulpecula* (Hocking, 1981). Maynes (1976) suggests that this period represents a continuation of differentiation and development of the embryonic organs. However, this initial growth stage in the ringtail also includes the rapid development of physical characteristics (i.e., limbs, lips opening, eyes opening etc.).

After the young have released the teat the relative growth rate in ringtail possum sucklings declines to a constant rate over the period during which the young emerge from the pouch. Hence, although the absolute mass increment of the young at this second stage of growth continues to increase, the relative rate of increase remains constant. This constant rate of growth may be because of increasing requirements for energy for the maintenance components of the young's energy budget (i.e., development of homeothermy and endothermy). This would partition nutrients and energy in the milk away from growth. In addition, changes in the

structure of the alimentary canal may occur immediately prior to pouch exit as found in other marsupial species (i.e., *Thylogale stigmatica* and *Thylogale thetis*, Langer, 1979; *Macropus eugenii*, Janssens, 1984, Janssens and Ternouth, 1987). Such changes are required to accommodate the alteration in milk composition (summarised in Green and Merchant, 1988) and the transition to an adult diet. This metamorphosis of the alimentary canal would also divert energy and nutrients away from growth.

During pouch life the young are growing in a controlled environment feeding on their mothers milk. The maternal environment may play a critical role in determining the growth rate of the young. This is illustrated in experiments involving the exchange of sucklings between different species by Merchant and Sharman (1966). They showed that the growth rate of young of one macropod species could be accelerated by transferring it to the pouch of a macropod species which showed a faster rate of growth. Similarly, Maynes (1973) found a reduction in the growth rate of a large pouch young transferred to a pouch which had previously been occupied by a smaller young.

After emergence from the pouch the absolute weekly mass increment continued to increase in ringtail possum sucklings. However, this increase was not as steady as that observed during pouch life and one peak in mean mass increment at 23 weeks of age was significant. The reasons for this peak is unclear although it corresponds to the time at which young were first noted to practice caecotrophy.

The young ringtail possum still grew relatively rapidly after it had emerged from the pouch although the rate of growth was relatively constant. During this second stage there is competition for nutrients from the diet between growth and energy requirements for activity and thermoregulation. The nutritional physiology and biochemistry of the young continues to change during this stage to enable complete transition to the adult foliage diet (Janssens and Messer, 1988). This would further partition nutrients in the diet away from growth. The rate of growth of the ringtail possum suckling declines again to a constant rate during the final weaning period. This represents the stage at which the young ceases to suck milk and the completion of its transition to the adult diet.

Changes in the rate of growth in the suckling is possibly influenced by changing quantity and quality of the mothers milk. However, the pattern of growth and development is influenced by the partitioning of dietary nutrients between actual

growth and maintenance (i.e., metabolic rate and thermoregulation). Growth will also be dependent on the rate of cell differentiation and multiplication (Ricklefs, 1979). Rose (1989b), suggests that this is the reason for the differences in embryonic growth rates between *Potorous tridactylus* and *Bettongia gaimardi*.

Values of instantaneous relative growth rate ('k') enable comparisons between species regardless of their body size. Rose (1989b) used values of 'k' to compare growth rates of macropodids during embryonic development and pouch life. However, limitations in the use of 'k' to compare growth rates of different species result from interspecific differences in the stages at which 'k' is constant.

Other comparative studies of growth rate in marsupial sucklings (Case, 1978 and Russell, 1982) have concentrated on comparison of absolute growth rate over a particular stage of growth. Russell (1982) estimated the growth rate (g/day) for the ringtail possum over the period from pouch exit to weaning from data collected by Thompson and Owen (1964). Her estimate suggests that the ringtail young grow slightly faster than the predicted rate for a marsupial. However, she assumed linear change in body mass during this period whereas this study has shown a quadratic growth curve for the change in ringtail sucklings' body mass from pouch emergence to weaning.

Lee and Cockburn (1985) argue that the studies by Case (1978) and Russell (1982) are of little significance when it comes to interspecific comparisons. Firstly, because they cover stages of growth which represent a larger proportion of the overall growth in some species than in others. Secondly, because allometric variation in absolute growth rate (g/day) has little ecological significance. They introduced the use of the Gompertz constant for interspecific comparisons of marsupial growth. The main advantage of this method is that it enables estimation of the rate of decay of growth over the complete growth trajectory. In addition, Cockburn and Johnson (1988) show that deviations from the general Gompertz growth model enables assessment of variations from the 'typical' pattern of growth.

The mean Gompertz constant estimated for ringtail possum young reared in captivity (i.e., 0.01) suggests that this species has a slow rate of growth for its body size when compared with the rate predicted allometrically from a range of marsupial species i.e., 0.014, (Lee and Cockburn, 1985). This is in contrast to another petaurid species, *Petaurus breviceps*, whose Gompertz constant suggests a fast rate of growth for its body size (Smith. M.J., 1979b in Lee and Cockburn, 1985).

The physiological mechanisms by which more rapid growth is accomplished is one aspect of the question as to why some animals grow faster than others (Case 1978). The slow growth rate of the ringtail possum may be due to a low rate of net assimilation of nutrients and/or a low efficiency in converting assimilated nutrients into body tissue (see Chapter 8). Oftedal(1984) found that low rates of growth in developing young of primate species correlated with low milk energy yield. The low available energy content of the ringtail possums foliage diet may lead to a relatively low rate of milk production by the mother (Russell, 1982; Tyndale-Biscoe, 1984, see Chapter 8). Furthermore, the low protein content of a foliage diet (Hladik, 1978; Cork and Pahl, 1984; Smith and Lee, 1984) may be reflected in the protein content of the milk which could subsequently result in low growth rates for the sucklings (Bjornhag *et al.*, 1979, see Chapter 7). However, another marsupial folivore, *Trichosurus vulpecula*, has a Gompertz constant which suggests a relatively high growth rate (Lee and Cockburn, 1984). Nevertheless, this may reflect the lower degree of folivory for this species (Pahl, 1984b; Eisenberg, 1978). Gompertz constants are not available for the highly folivorous arboreal marsupials e.g., *Petauroides volans* and *Phascolarctos cinereus*. Nevertheless, Russell (1982) has shown that rate of development for these species is slow relative to other arboreal species.

How *et al.*, (1984) noted intraspecific differences in growth rate of the head for ringtail possum pouch young and suggested that they were related to the nutritional plane of the mother. These differences appear to occur within a population as well as between distinct populations which suggests that milk production by mothers within a population may vary between different years. Hence, intra-specific differences in growth rates are probably adaptations to different microhabitats instead of different geographical ranges.

3.4.4 Comparison of field and captive growth

Growth of the head and development of wild *P.peregrinus viverrinus* appears to parallel growth and development of captive young from birth to weaning. This contrasts with two studies comparing growth of marsupial young between the field and laboratory (*Macropus robustus*, Ealey, 1967; *Dasyurus geoffroii*, Serena and Sodequist, 1988). These studies indicate a faster rate of growth in captive

young during late pouch life. However, other studies have found no difference in the rate of growth between field and captive marsupial sucklings (*Macropus eugenii*, Murphy and Smith, 1970; *Bettongia gaimardi*, Taylor and Rose, 1987). The latter study noted no difference in growth rate of the pes in field and laboratory pouch young, however, they did note that captive young were heavier than field young toward the end of pouch life. Similarly, the only difference noted between field and captive ringtail possums in this study was a slightly higher mass at final weaning for captive young (473.5 ± 51.5 , $n=13$) compared with that obtained for field young (359 ± 109.8 , $n=11$) (Table 3.15). Hence although the young had the same skeletal growth rates the young in captivity appear to be in better condition.

Serena and Sodequist (1988) found that wild growth of *Dasyurus geoffroii* was dependant on maternal 'condition' and hence suggested that variation in growth reflects variation in the quantity or quality of maternal diet. These differences or similarities in rate of growth or condition of the young appear to reflect the differences or similarities in the nutritional plane of the field or captive mother. The similar rates of growth for the ringtail possum in this study suggests that there was little difference in the milk between field and captive mothers (see Chapter 7). However, the lower condition of wild animals, as indicated by their body mass at weaning, may reflect the extra energy costs of free existence for these young after they leave the pouch.

The duration of lactation in the majority of wild *P. peregrinus viverrinus* was similar to that of captives although using the criteria outlined earlier in this chapter, it was difficult to determine the exact cessation of lactation in the field. Few studies have compared the length of lactation in animals maintained in captivity with those in the wild. Green *et al.* (1987) found *Dasyurus viverrinus* suckled for longer in captivity than in the wild and suggested that conditions of close confinement may have prolonged lactation. However, the duration of lactation in the Tasmanian devil, *Sarcophilus harrisii*, is longer in the wild (Guiler, 1970; D. Pemberton, personal communication.) than in captivity (Fleay, 1952). Similarly, one wild ringtail possum in this study was lactating at 31 weeks post partum which suggested that in this particular female the duration of lactation was closer to eight months than seven months. Russell (1982) proposes that the ready availability of food in captivity could lead to earlier weaning.

3.4.5 Reproduction

The reproductive traits of the population of ringtail possums on Flinders Island show some similarities and some differences to those of other populations described in the literature (Thompson and Owen, 1964; Marsh, 1968; Hird, 1975; How *et al.*, 1984; Pahl and Lee, 1988, see Table 3.16). The main autumn/winter birth season for the ringtail possums on Flinders Island was similar to that reported for other populations on mainland Australia (see Table 3.16). The timing of these births means that the young emerged from the pouch and began eating leaves during the spring and early summer months. A similar situation has been noted for other marsupial folivores (*P.cinereus*, Eberhard, 1978, Martin and Lee, 1984; *T.vulpecula*, How, 1981, Kerle, 1984, Hocking, 1981; *T.caninus*, How, 1976, How, 1981; *P. volans*, Smith, 1969, Henry, 1984). Young leaves are the preferred food of ringtail possums (Pahl, 1984) and are most abundant during the spring and summer months in Victoria (Pahl, 1987b). Similarly, young foliage was observed to be most abundant at Whitemark beach and Paddies during the spring and summer months (see Chapter 2). Pahl and Lee (1988) argue that the timing of reproduction in the ringtail possum is related to the seasonal availability of young leaves. Such leaves contain more nitrogen and water but less fibre than the more mature leaves (Cork and Pahl, 1984; Pahl and Hume, 1988 in Pahl and Lee, 1988). As mentioned earlier the digestive physiology of the young ringtail possum is probably still adapting to its new leaf diet during the period between pouch emergence and weaning. Therefore an abundant supply of nutritious young leaves at this stage is important for the young which is still growing rapidly.

Three other studies report a second peak of births during the spring and early summer months (How *et al.*, 1984; Pahl and Lee, 1988; Marsh, 1968). These births are mainly restricted to multiparous females breeding twice in a year (Pahl and Lee, 1988). However, this second peak was not so obvious in the population on Flinders Island with only a small proportion of multiparous females (14%) rearing a second litter. This fact and the lower mean litter size resulted in a low fecundity for the population on Flinders Island compared with that of populations at Lysterfield and Sandy Point in Victoria (see Table 3.16). Similarly, Thompson and Owen (1964) recorded few cases of a female rearing two litters in a year and a low mean fecundity in the population at Warramate, Victoria.

A similar variation in the occurrence of a secondary breeding period is found

Table 3.16 Reproduction in the common ringtail possum.

Site	Age at sexual maturity (months)	Mass at sexual maturity (g)	Litter size	Main Birth season	Fecundity Young/female /year	References
Warramate (Victoria)	12	629.3	1-3	May-Nov	1.8	Thompson and Owen (1964)
Sydney (N.S.W)	18	-	1-3	May-Sept	2-4	Marsh (1968)
Sandy Point (Victoria)	13-14	630	1-3	April-Aug	2.35	How <i>et al.</i> , (1984)
Sandy Point (Victoria)	-	643	1-3	May-Aug	-	Hird (1975)
Lysterfield (Victoria)	12.1	636	1-4	May-July	2.36	Pahl and Lee (1988)
Sandy Point (Victoria)	12.9	665	1-3	May-July	2.20	Pahl and Lee (1988)
Flinders Island (Tasmania)	13	895	1-2	May-Aug	1.91	This study

in studies of different geographic populations of the brushtail possum (e.g., Dunnet, 1964; Crawley, 1973; Hocking, 1981). In addition, Hocking (1981) noted a variation in the breeding potential of brushtail possum populations between forest habitats of different ages after fire. The highest fecundity was recorded for animals living in younger habitats (recently burnt) where the nutrient content of the vegetation was considered highest (Hocking, 1981). Other studies have noted correlations between fecundity and the nutritional status of a population (reviewed in Sadleir, 1969). The nutrient status of vegetation may decline with age (Attiwill, 1980). Therefore, the lower fecundity recorded for the population of ringtail possums on Flinders Island may be related to the mature nature of their habitat (see Chapter 2) compared with the younger stand of coast teatree at Sandy Point where annual fecundity was relatively high (Pahl, 1987b; Pahl and Lee, 1988).

Pahl and Lee (1988) note a greater mass for weaned young reared by females which breed twice in a year compared with those of single breeders. They tentatively suggest that this was related to the availability of food for individual lactating females. A higher nutrient intake by a lactating female may be reflected in the composition of the milk which in turn could increase the growth rate or condition of the young. This would explain the shorter duration of lactation noted for females which breed twice in a year (Pahl and Lee, 1988; this study). However, another factor that may influence the opportunity for multiparous females to breed for a second time may be the availability of males. Some male ringtail possums are able to reproduce at one year after their birth (How *et al.*, 1984; this study). The higher proportion of spring and summer births in the population at Sandy Point compared with the Flinders Island population would result in a higher proportion of young males reaching maturity in the following spring/summer months. Further work is needed on reproduction in the male ringtail possum to determine factors controlling the onset of breeding.

3.4.6 Body mass and condition of adults

The seasonal variation in body mass for adult male and female ringtail possums on Flinders Island was similar to the pattern noted for the populations at Sandy Point (How *et al.*, 1984; Pahl, 1987a) and Lysterfield (Pahl, 1987a). The females tended to put on weight during early lactation (Phase 2a and Phase 2b),

when the young were suckled in the pouch. They lost weight during the late spring and summer months when most of them were suckling back young (Phase 3 of lactation). Therefore, the majority of females were lightest during late autumn when mating commenced. The population at Warramate showed a slightly different pattern in seasonal body mass change, however, the females still tended to lose weight at a time which coincided with late lactation (Thompson and Owen, 1964). The identical mass and skeletal development of male and female ringtail possums, and the lack of sexual dimorphism in adults is similar to that of monogamous primate species (Clutton-Brock *et al.*, 1977). Perhaps the lack of sexual dimorphism in the ringtail possum reflects its social behaviour.

The body mass of adult ringtail possums in this study was generally higher than that recorded for adults in other populations (Table 3.16; Thompson and Owen, 1964; Hird, 1975; How *et al.*, 1984; Pahl, 1985). The mean monthly body mass of females at Sandy point in Victoria, which show a similar seasonal variation in body mass to adult animals on Flinders Island, ranged from 684g to 815g (Pahl and Lee, 1988), whereas those on Flinders Island ranged from 948g to 1082g. In addition, the body mass of primiparous females suckling their first litter (measured within a month of the estimated date of birth for the litter) on Flinders island was higher than that reported by Pahl and Lee (1988) for primiparous females at Sandy Point (respectively: 896 ± 89 , $n=9$; 665 ± 56 , $n=32$). The age at which primiparous females produced their first litter on Flinders Island was estimated to be approximately 13 months of age which was similar to that noted for those females at Sandy point (Pahl and Lee, 1988). This in conjunction with the slightly higher mass at weaning (see earlier) suggests a higher absolute growth rate for the ringtail possums on Flinders Island. A larger body size and different growth pattern has also been noted between the Tasmanian brushtail possum and those found on mainland Australia (Hocking, 1981).

Many mammals follow Bergmann's rule (Bergmann, 1847 in Barnett, R. J, 1977) and increase in body size at higher latitudes (Case, 1978). It appears that the ringtail possum is no exception. Difference in body size of populations of possums that are geographically distinct may reflect underlying genetic variation. Tasmanian ringtail possums, geographically isolated from mainland Australia when the climate was cooler, may have evolved a larger body size allowing them to cope with the cold (Kendeigh, 1969). Alternatively, the larger body size of ringtail possums in

Tasmania may reflect the absence of the other strictly folivorous species (i.e., *Phascolarctos cinereus* and *Petauroides volans*) and hence an increased size of available food resource (McNab, 1971).

CHAPTER 4

ACTIVITY PATTERNS AND DIET OF THE COMMON RINGTAIL POSSUM, IN A *Leptospermum laevigatum*, THICKET IN TASMANIA.

4.1 Introduction

Studies in Victoria (S.E Australia) have shown that the ringtail possum feeds on foliage from a wide variety of trees and shrubs, particularly those belonging to the Myrtaceae (Thompson and Owen, 1964; Pahl, 1984; Pahl, 1985; Pahl, 1987b). Such foliage is regarded as a poor food source due to a low available caloric density resulting from its high fibre content and allelochemicals (Cork and Pahl, 1984, see Chapter 1). The low available caloric density of foliage has lead to the suggestion that arboreal folivores may conserve energy to a greater extent than other mammals by minimising activity and other maintenance costs (Eisenberg, 1978; McNab, 1978; Hume *et al.*,1984). The howler monkey, *Alouatta palliata*, is one example of a folivorous arboreal mammal whose behaviour appears to be consistent with this hypothesis: they spend a large proportion of their time resting and their temporal pattern of activity shows little variation (Milton, 1978). Marsupial folivores studied to date (*Petauroides volans*, Kehl and Borsboom, 1984; *Trichosurus vulpecula*, MacLennan, 1984; *Phascolarctos cinereus*, Smith, 1979b) show similar conservative activity budgets. However, there have been no published studies of the behaviour and/or activity patterns of the nocturnal ringtail possum.

Energy for reproduction and storage must be accumulated in excess of energy for basal metabolism, thermoregulation, growth, feeding and other activities. Mammals may accomodate energy demand during reproduction by increasing the quantity of energy assimilated, utilizing assimilated energy stored in the body and/or by reducing energy expenditure on some component of the energy budget (Racey and Speakman, 1987). Use of one or more of these strategies may be reflected in the particular behaviour and activity pattern of an animal. For example, a study of the South American marsupial, *Caluromys philander*, found that females suckling young older than 5 weeks were active longer than non-lactating females (Atramentowicz, 1982). This difference in nocturnal activity was related to the

increased food requirement of females suckling large young. Similarly, the accumulation of energy by, *Marmota flaviventris*, to supply the costs of reproduction, is reflected in an increase in the length of foraging bouts by reproductive females (Melcher *et al.*, 1989). In addition, these females also appear to minimise energy expenditure for thermoregulation and reduce the time spent on miscellaneous behaviours (Melcher *et al.*, 1989). Reduced locomotor activity during reproduction has been noted in *Sigmodon hispidus*, and this may partially compensate for the extra energy costs of reproduction (Randolph *et al.*, 1977). Furthermore, small insectivorous bats may minimize energy expenditure during lactation by entering torpor (Racey and Speakman, 1987).

This study examines the temporal patterns of feeding and other behaviours exhibited by free-living ringtail possums and the possible influence of reproduction on the allocation of time to particular behaviours. Feeding is examined more closely to provide a qualitative estimate of the diet of ringtail possums in the *Leptospermum laevis* thicket on Flinders Island, Tasmania. Chemical analyses of the leaves eaten by the possums were undertaken to provide insight into the proportion of dietary compounds a ringtail possum, living in tea tree scrub, may encounter. Such information, on the diet and chemical composition of the diet, will enable conversion of field metabolic rate measurements to energy units and subsequent estimation of food consumption by free-living ringtail possums (see Chapters 5 and 6).

4.2 Methods

4.2.1 Study area and animals.

Observations of sixteen adult ringtail possums (ten females and six males) caught at Whitemark Beach were conducted between June 1986 and June 1988. Information on nest occupancy was collected during the capture-mark-release programme at Paddies and Whitemark Beach (see Chapter 2). Additional information on the frequency of changes in nest site was collected from thirty-seven animals (nineteen females and eighteen males) at Whitemark Beach. The study area, general capture and handling techniques have been described in Chapter 2. Methods used to determine the reproductive status of the adults are given in Chapter 2 and 3.

4.2.2 Activity observations

Field Methods (radio-telemetry)

Individual ringtail possums were fitted with radio transmitters (tuned loop brass collar tags, Biotrack, U.K.) which enabled animals to be located in their nest sites during the day and subsequently identified and followed at night. The radio signal was monitored using a Customs Electronics CE 12 receiver in conjunction with a portable 2 element Yagi antenna (Fig 4.1a). The transmitters had a range of 400-600 metres to animals on the ground and 1-3 km to animals in the trees. However, the maximum distance at which the radio signal could be received varied according to topography.

Radio-transmitters were fitted to captive animals prior to their use in the field to assess their effect on the behaviour of the possums. Individual possums quickly adapted to their radio-collar and made few attempts to remove it. Many radio-telemetry studies, including those on the badger *Meles meles* (Cheeseman and Mallinson, 1980), the striped skunk *Mephitis mephitis* (Sargeant, 1980) and the native quoll *Dasyurus viverrinus* (Godsell, 1983) highlight the importance of collar fit. Tight collars may cause skin abrasions and loose collars can result in the animal dislodging the collar or snagging a forepaw. Care was taken to ensure an optimum collar fit around the neck of each individual ringtail possum before it was released. Throughout the whole study only one collar was dislodged during capture and no animals suffered neck abrasions.

The individual radio-collars weighed 20-25 g which approximated 2% of the adult ringtail possums body mass and was well within the 5% limit suggested as a maximum weight by Macdonald (1978). However, this maximum collar weight is only an informal standard which has not been evaluated for mammal species. Gessaman and Nagy (1988a) found homing pigeons, *Columba livia* fitted with harnesses and transmitters weighing either 2.5% or 5.0% had to work harder and longer during a long distance flight. Although recapture of individual ringtails without radiocollars was difficult, a few successful attempts enabled the metabolic rate of free-living ringtails with and without radio-collars to be assessed (see Chapter 5).

Each possum carried a radio-collar for 5-6 days. After this period the animal was radiotracked to its nest during the day, caught by hand (see Chapter 2) and the

collar was removed until the next fieldtrip. The activities of two to three individual possums were monitored on each fieldtrip. Some months' radio-collared sample included animals which had carried radio-collars on previous fieldtrips, as well as newly collared animals. Radio-surveillance techniques were used, i.e., the animal was located by radio-tracking and was then observed for up to six hours. Some animals were radio-tracked to their nest during the day and then followed after they had emerged from the nest at dusk. The time at which these animals emerged from the nest was recorded. Whilst following the animal, its behaviour was recorded continuously. The time at each behaviour change and any tree species eaten was noted.

This direct observation of the possums was aided by Beta lights (Biotrack, U.K) fitted to the base of the transmitter under the possums chin (see Fig 4.1). These green lights did not appear to interfere with the animals night vision. They enabled the possum to be visually located without the need to use the radio receiver, thus minimising noise which might disturb the animal.

The animal's particular behaviour was noted with the aid of binoculars. When necessary, a portable spotlight masked with a red filter was used to confirm the activity of the possum and identify the tree species. Dense foliage frequently obscured the view of the animal and on these occasions sound was relied upon to determine its behaviour (e.g., feeding was recognised by a 'snap-munch-munch-munch' sound). Every effort was made to prevent observer disturbance to the animal and all recordings were made by the author.

The behaviour of a surveyed animal was assigned to one of the following categories:

- (1) Feeding,
- (2) Moving - moving from one location to another, excluding movements in a tree associated with foraging,
- (3) Stationary - remaining immobile in one location
- (4) Interacting with another possum (including vocalisations between possums),
- (5) Grooming,
- (6) 'In nest' - when a surveyed animal entered and remained in a nest during the observation period.

A total of 111.10 hours of observations were made, during 31 nights of 3-6 hrs

(a)



(b)



Figure 4.1 (a) Radio-tracking ringtail possums in a *Leptospermum laevigatum* thicket.
(b) A free-living ringtail possum wearing a tuned loop brass collar tag with Beta light.

continuous surveillance.

In addition to continuous surveillance, point fixes of individual animals throughout the night (i.e., location of the animal every hour and monitoring for up to 15 mins to determine if active or inactive) were attempted. However, problems associated with this technique resulted in it being abandoned. Firstly, the noise associated with radio-tracking the animal, each hour, in dense tea tree scrub resulted in considerable disturbance to the possum. Secondly, although location of the transmitter on the ringtail in a nest during the day was relatively easy, location of the active animal at night in dense bush proved difficult.

Analytical Methods

Monthly observations of activity were pooled by season (Spring/Summer or Autumn/Winter). The time allocated to each behaviour was expressed as a percentage of the total hours of observed behaviour for each season. In addition, observations of activity were pooled by sex of the animals. The female sample was further divided according to reproductive status (non-lactating, suckling pouch young or suckling back-young). The time allocated to each behaviour was also expressed as a percentage of the total hours of observed behaviour for each animal group. Time spent out of sight (not including the time animals spent in a nest) was omitted from the totals, as was any behaviour that resulted from observer-disturbance.

MacLennan (1984) showed that the proportion of time spent on a particular behaviour by *Trichosurus vulpecula* varied considerably at different times of the night. Therefore, the comparisons of the proportion of observed activity time spent on a particular behaviour for ringtail possums in this study may be biased according to the number of observations made at a particular time of night. However, attempts were made to obtain observations at different parts of the animal's active period, so that pooled seasonal results and group results included observations made at all periods of the night.

A qualitative estimate of the diet of ringtail possums in the study area was made from the percentage of total feeding time spent feeding on a particular tree species. This was done for the total feeding observations and for those pooled according to season.

4.2.3 Nest Occupancy and Nest Site Movement.

Daily location of radio-collared animals in their nests over a five day period was used to provide information on the frequency of changes in nest site by a particular animal. Located animals included those surveyed at night and the remaining animals fitted with radio-collars for the study of field metabolic rate (see Chapter 5). This information was collected on 47 occasions between October 1986 and January 1988. The results were pooled by the sex of the animals and reproductive status of the females.

The number and sex of nest companions were recorded throughout the capture-mark-release programme (see Chapter 2). This enabled a seasonal comparison of the composition of nest groups to be made. The following nest group categories were used;

- F - female alone,
- M - male alone,
- MF - male and female,
- FF - two females,
- MM - two males,
- MMF - two males and a female,
- FFM - two females and a male,
- FMFM - two females and two males,
- MFMM - three males and a female,
- MFFF - three females and a male,

Number of nests in each category were expressed as a proportion of the total number of nests examined each month. Dependent young ('back-young' see Chapter 3) were not included in the analysis. Nest groups which included females were examined according to the females reproductive status.

4.2.4 Chemical Composition of Leaves

Samples of foliage from the major tree species in the study area were collected between 3.00 pm and 5.00 pm during April 1987, June 1987, September 1987, December 1987 and January 1988. Leaves which had their petioles attached were stripped from peripheral branches of trees chosen at random. Each sample,

which contained a mixture of young and old leaves, was placed in a sealed plastic container and transported to the laboratory in a cool-box. In the laboratory each sample of leaves was weighed to the nearest 0.01 g on a Mettler PE 3600 balance, dried in an oven at 50°C for 24-36 hrs. and then stored at -20°C. Prior to chemical analysis, subsamples were ground with a pestle and mortar (for dry matter and energy content determinations), or through a 1 mm screen in a centrifugal mill (for organic matter, total nitrogen, crude lipid, total phenolics and cell wall constituent analyses).

Dry Matter

Dry matter content was determined by drying the leaf samples at 80°C in a force draught oven to constant mass (36-48hrs).

Organic Matter

Samples were ignited in a muffle furnace at 530°C for 3 hrs and the percent organic matter was determined as:

$$\text{organic matter (\%)} = \frac{\text{leaf dry mass} - \text{ash mass}}{\text{leaf dry mass}} \times 100$$

Gross Energy

Ground dried leaf samples were compacted into discs and then ignited in a Gallenkamp Ballistic Bomb Calorimeter. The gross energy content (kJ/g) of each leaf species was then determined from the heat of combustion using benzoic acid as a standard.

Total nitrogen

Total nitrogen was determined by K Newgrain, Division of Wildlife and Rangelands Research, C.S.I.R.O, Canberra using the semi-micro Kjeldahl method (AOAC, 1970). Selenium dioxide and copper sulphite were used as the catalyst and ammonia was measured by titration. The crude protein content of the leaves was estimated as:

$$N (\% \text{ dry leaf mass}) \times 6.25 \text{ (Cork } et al., 1983).$$

Crude Lipid

Crude lipid content was estimated by K Newgrain, Division of Wildlife and Rangelands Research, C.S.I.R.O, Canberra. The Soxhlet extraction method was

used with petroleum ether as the solvent. The lipid extracted was measured by weight loss of extracted sample.

Total Phenolics

Total phenolics were determined following the method of Cork and Pahl (1984), modified by S. Cork (personal communication). Known weights of dried and ground leaf samples were extracted in 50% acetone and total phenolics were determined spectrophotometrically using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Gallic acid (75 mg in 50 mls of 50%MeOH) was used as a standard.

It should be noted here that Cork *et al.*, (1983) found that oven dried leaf samples were lower (approx. 11%) in total phenolics than fresh leaf samples. However, it was not feasible to analyse fresh leaves in this study.

Cell-wall Constituents

Known weights of the dried and ground leaf samples (0.5 g) were extracted for three days in 50% methanol for removal of phenolics. To determine total cell-wall constituents the phenolic-free residues were subjected to neutral-detergent extraction as Goering and Van Soest (1970) but omitting sodium sulphite (Cork and Pahl, 1984). To determine acid-detergent fibre and acid lignin, separate residues were sequentially extracted in neutral-detergent, acid-detergent and 72% sulphuric acid following the procedures of Goering and Van Soest (1970), modified by Cork and Pahl (1984). Cellulose was determined as the difference between total acid-detergent fibre and acid lignin. Hemicellulose was determined as the difference between total cell wall constituents and acid-detergent fibre.

4.2.5 Statistical Methods.

Means \pm standard deviation are presented. Chi-square analysis for independence was used to compare the proportions of activity time spent in each behaviour by males, females and females of different reproductive status. Comparisons between two means were made using Student's t-tests (un-paired and two-tailed unless otherwise stated). Percentages were transformed using the arcsine transformation and a square root transformation was used for counts of nest site changes to improve normality of the data. The 0.05 level of probability was accepted as indicating statistical significance.

4.3 Results

4.3.1 Activity

Ringtail possums left their nests on average 32.6 ± 25.6 minutes ($n = 19$) after sunset throughout the year. Three animals were observed entering their nests at the end of their active periods 34.3 ± 14.1 minutes before sunrise. In general, after leaving the nest the observed animal moved to a nearby tree and commenced feeding in the canopy. Females with back-young tended to leave the young on their own either in the nest or feeding on the nest tree whilst the mother moved to feed a short distance away.

It was noted that the majority of defecation by the animal occurred during the initial period of feeding. Particular trees used regularly by animals were easily identified by the faecal pellets which accumulated beneath them. Feeding bouts were followed by periods of 'stationary' behaviour. This stationary period occasionally included periods of grooming. Only autogrooming was observed and most observations involved females grooming their pouch.

Observed interactions between individual animals involved;

- (1) a member of the opposite sex approaching the observed animal, while calling frequently (see Appendix B) after which the two animals would move to a nearby tree and commence feeding.
- (2) a female suckling a back-young whilst poised on a branch.
- (3) agonistic encounters, during which an individual disturbed the observed possum whilst it was 'stationary'. The observed animal chased the intruder from the tree and after a moment of grappling the intruder fell from the tree limb to the ground. On recovering from the fall the intruder hastily retreated.

The major proportion of observed activity time was allocated to feeding (34 - 44%). Observations of 'stationary' behaviour also accounted for a significant percentage of the time (28%). Moving from one position to another represented 21 - 22% of activity and the remaining time was made up by interactions with other possums, grooming behaviour and time spent in the nest (Figure 4.2).

Chi-square analyses showed that observed differences in the proportion of time spent on each behaviour by animals during the Autumn/Winter and

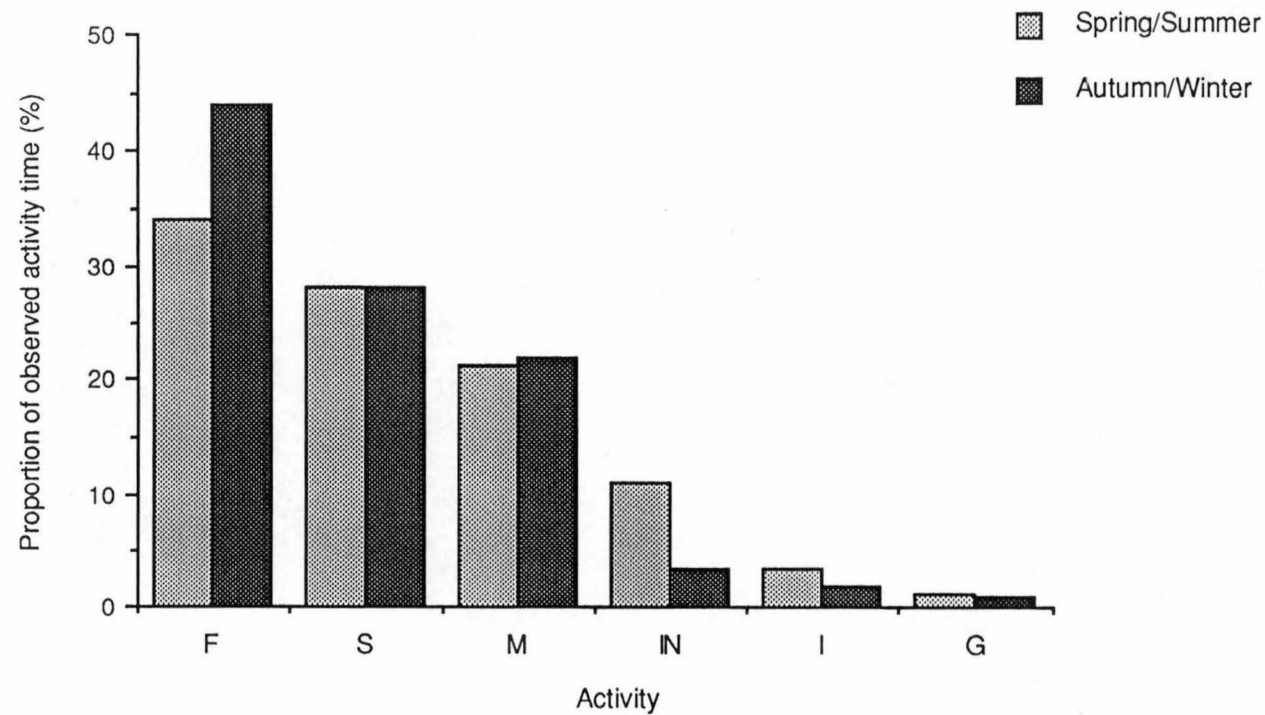


Figure 4.2

Percentage behaviour classes comprising total activity observed during Spring/Summer months and Autumn/Winter months.

F = feeding, S = stationary, M = moving, I = interacting, G = grooming, IN = in nest

Table 4.1 Comparison of the proportions of observed activity time(minutes) spent on particular behaviours by ringtail possums during Spring/Summer (S/S) and Autumn/Winter (A/W). The 'immobile' category is an aggregate of the 'stationary' and 'in nest'categories. (Chi-square contingency table, O = observed, E = expected)

Season	Feeding	Moving	Immobile	Grooming	Interacting	Total
S/S	O=1103	O=663	O=1213	O=36	O=107	3122
	E=1245	E=675	E=1087	E=34	E=81	
	$\chi^2=16.3$	$\chi^2=0.21$	$\chi^2=14.6$	$\chi^2=0.1$	$\chi^2=8$	
A/W	O=1556	O=778	O=1109	O=36	O=65	3544
	E=1414	E=766	E=1235	E=38	E=91	
	$\chi^2=14.3$	$\chi^2=0.2$	$\chi^2=12.8$	$\chi^2=0.1$	$\chi^2=7.4$	
Total	2659	1441	2322	72	172	6666

Table 4.2 Comparison of the proportions of observed activity time (minutes) spent on particular behaviours by male and female ringtail possums. The 'immobile' category is an aggregate of the 'stationary' and 'in nest' categories. (Chi-square contingency table, O = observed, E = expected)

Sex	Feeding	Moving	Immobile	Grooming	Interacting	Total
Male	O = 639	O = 235.0	O = 467	O = 0	O = 30.0	1371
	E = 547	E = 296.4	E = 478	E = 14	E = 35.4	
	$\chi^2 = 15.5$	$\chi^2 = 12.7$	$\chi^2 = 0.25$	$\chi^2 = 14$	$\chi^2 = 0.83$	
Female	O = 2020	O = 1206	O = 1855	O = 72	O = 142	5295
	E = 2112	E = 1144.6	E = 1844	E = 57.2	E = 137	
	$\chi^2 = 4.0$	$\chi^2 = 3.3$	$\chi^2 = 0.1$	$\chi^2 = 3.8$	$\chi^2 = 0.2$	
Total	2659	1441	2322	72	172	6666

Table 4.3 The proportion of total observed activity time spent on particular behaviours by adult ringtail possums.

Adult Animal Group	Proportion of observed activity time spent (%)						Sample size (hrs)
	Feeding	Moving	Stationary	Grooming	Interacting	In nest	
Non-lactating Females (n=10)	35.90	25.90	31.10	1.30	2.30	3.4	31.55
Lactating Females: Phase 2a and 2b (n = 8)	31.05	23.49	30.43	2.14	2.97	12.9	31.56
Phase 3 (n = 5)	50.30	17.80	16.50	3.40	2.70	12.3	24.24
Males (n = 6)	46.60	17.10	32.60	0.00	2.20	1.5	22.51

Table 4.4 Comparison of the proportions of observed activity time (minutes) spent on different behaviours by females of different reproductive status. The 'immobile' category is an aggregate of the 'stationary' and 'in nest' categories. (Chi-square contingency table, O = observed, E = expected)

Female group	Activity Category					Total
	Feeding	Moving	Immobile	Grooming	Interacting	
NL	O = 688	O = 496	O = 660	O = 26	O = 45	1915
	E = 730.6	E = 136.1	E = 671	E = 26	E = 51	
	$\chi^2 = 2.48$	$\chi^2 = 8.30$	$\chi^2 = 0.18$	$\chi^2 = 0$	$\chi^2 = 0.7$	
Phase 2a and 2b	O = 595	O = 450	O = 773	O = 41	O = 57	1916
	E = 730.9	E = 436.4	E = 671.2	E = 26	E = 51.4	
	$\chi^2 = 25.3$	$\chi^2 = 0.42$	$\chi^2 = 15.4$	$\chi^2 = 8.65$	$\chi^2 = 0.6$	
Phase 3	O = 737	O = 260	O = 422	O = 5	O = 40	1464
	E = 558	E = 333.4	E = 512.8	E = 19	E = 39.3	
	$\chi^2 = 57.4$	$\chi^2 = 16.2$	$\chi^2 = 16.1$	$\chi^2 = 10.3$	$\chi^2 = 0.01$	
Total	2020	1206	1855	72	142	5295

Table 4.5 The estimated total actual time (hrs/day) spent during the night on particular behaviours by adult female ringtail possums.

Adult Female Group	Estimated actual time (hrs/day) spent on:						Total
	Feeding	Moving	Stationary	Grooming	Interacting	In nest	
Non-lactating Females	3.7	2.70	3.2	0.13	0.24	0.35	10.3
Lactating Females:							
Phase 2a and 2b	4.1	3.10	4.0	0.28	0.40	1.70	13.5
Phase 3	4.9	1.73	1.6	0.33	0.26	1.19	9.7

Spring/Summer months were highly significant ($\chi^2_4 = 74$, $P < 0.01$; Table 4.1). Animals spent more time 'immobile' during the Spring/Summer months due to a higher proportion of time spent in the nest (Figure 4.2). In addition, animals spent more time interacting with other possums but, less time feeding at this time of year (Table 4.1, Figure 4.2).

There were highly significant differences between males and females in the proportion of time allocated to each behaviour ($\chi^2_4 = 54$, $P < 0.01$; Tables 4.2 and 4.3). Males fed more than females but moved less. In addition, no males were observed grooming. The relatively small sample size (i.e., total observation time, Table 4.2) may have contributed to the lack of observations of grooming by males.

Differences were also observed between females of different reproductive status in the proportion of time allocated to particular behaviours (Table 4.3). Chi-square analyses showed that these differences were highly significant ($\chi^2_8 = 162$, $P < 0.01$; Table 4.4). Females suckling back-young (Phase 3 of lactation) spent proportionally more time feeding than females suckling pouch young (Phases 2a and 2b of lactation) and non-lactating females. The difference in the proportion of active time spent feeding was traded off against 'moving', 'stationary' and 'grooming' activities with females suckling back-young spending less time on these activities than females suckling pouch young (Table 4.3). Both groups of females suckling young spent a larger proportion of the observed activity time in the nest during the night than non-lactating females (Table 4.3).

Females were generally non-lactating between February/March and April, suckling pouch young from May to September and suckling back-young from September to January (Chapter 3). Assuming females left the nest 30 minutes after sunrise and returned to it 30 minutes before sunset (see earlier) the length of time each group of females spent nocturnally active was estimated to be 10.3, 13.5 and 9.7 (hrs/day), respectively (see Chapter 2 for sunset and sunrise times). Assuming the proportion of observed activity time represents the proportion of total nocturnal activity time, the actual time spent on each behaviour (hrs/day) by each group of females can be estimated (see Table 4.5). In addition, to a higher proportion of time spent feeding by Phase 3 females these females also spent approximately 1 hour more actual time feeding than non-lactating females.

Table 4.6 Seasonal variation in the composition of nest groups at Whitemark Beach.

Month	Proportion (%) of occupied nests examined with the following group composition:-										Total no. of nests examined
	F	M	MF	FF	MM	MMF	FFM	FMFM	MFMM	MFFF	
January	44.4	40.7	-	11.1	-	3.7	-	-	-	-	27
February	63.2	63.2	15.8	5.3	5.3	-	10.5	-	-	-	19
Mar/April	25.6	27.9	30.2	2.4	-	7.0	2.3	2.3	-	2.3	43
May	18.7	15.6	40.6	3.1	-	6.3	12.5	-	3.1	-	32
June	35.0	20.0	40.0	-	-	-	5.0	-	-	-	20
July	29.2	25.0	33.3	8.3	-	-	4.2	-	-	-	24
August	2.2	25.0	25.0	-	-	5.6	5.6	-	-	-	18
September	6.1	35.5	38.7	-	-	3.2	6.4	-	-	-	31
October	52.9	17.6	26.5	-	-	2.9	-	-	-	-	34
November	52.6	21.0	10.5	10.5	5.3	-	-	-	-	-	19
December	41.1	35.7	14.3	1.8	-	1.8	5.4	-	-	-	56
All	36.0	30.0	23.0	3.4	0.6	2.8	4.6	0.3	0.3	0.3	323

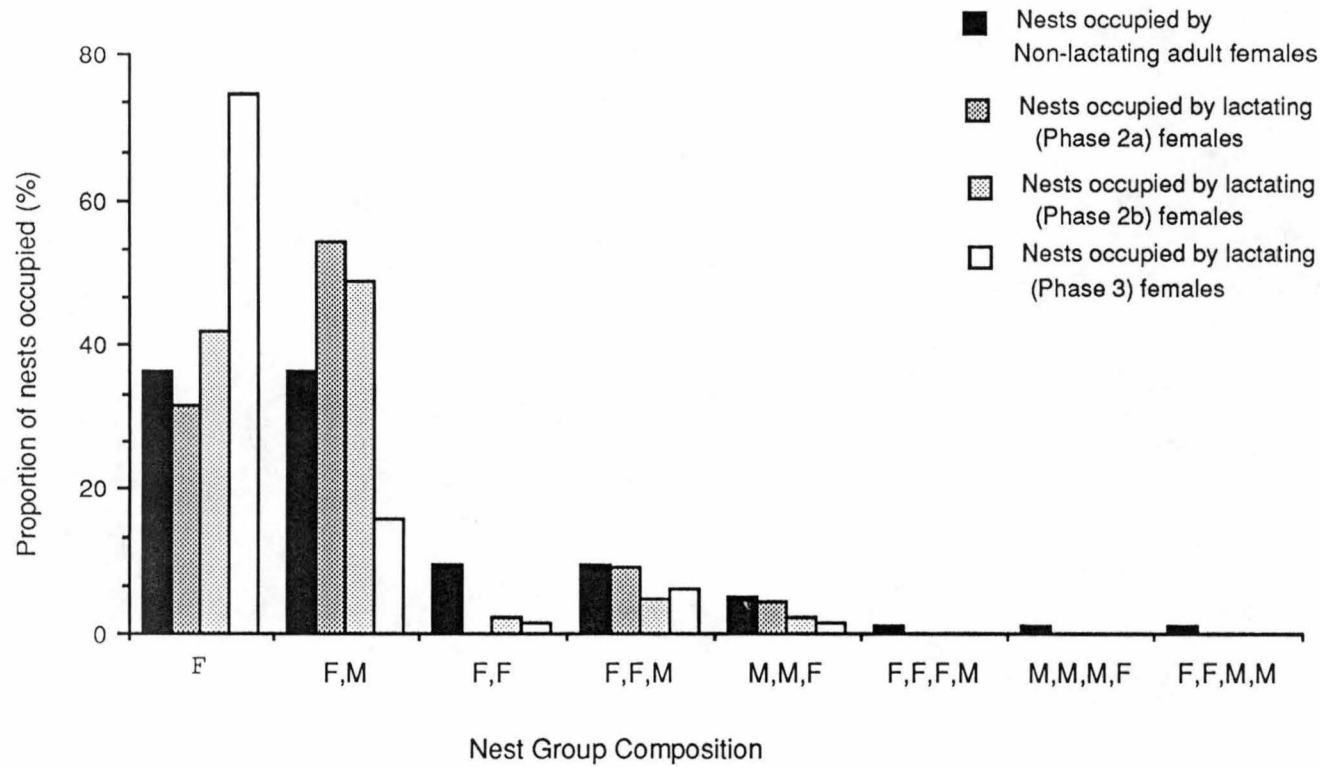


Figure 4.3

Variation in nest group composition according to the females reproductive condition. F = female, M = Male.(see text)

Table 4.7 Frequency of nest site movements over a five day period. Means \pm s.d and n are presented.

Adult Animal group	Number of changes in nest sites		
	Mean	SD	n
Males	3.1	0.8	23
Non-lactating Females	2.9	0.7	10
Lactating Females:			
Phase 2a and 2b	2.4	0.9	7
Phase 3	1.7	0.5	6
All Females	2.5	0.9	24

4.3.2 Nest Occupancy and Nest Site Movement

A total of 323 observations of nest group composition were made throughout the study (Table 4.6). No nests were observed to be occupied by more than four individuals (excluding back-young). Single individuals and male/female pairs formed the dominant nest groups throughout the year (Table 4.6).

The proportion of examined nests occupied by a single female was greatest during the late spring and summer months (41 - 63%) and lowest during August and September (2% and 6% respectively). The proportion of nests occupied by single males did not show as much seasonal fluctuation as that for individual females with the highest percentage of nests occupied by single males found during the summer months (36 - 63%).

The percentage of male/female pairs was highest (25 - 41%) during May and June and remained relatively stable throughout the winter and into early Spring. No nests examined during January were occupied by male/female pairs.

Only two nests were found to be occupied by two males. Both these nest groups consisted of an adult and a juvenile. Similarly, the two female nest group always consisted of an adult and a juvenile. In many cases this juvenile was identified as the female's offspring from the previous year. One young female was found with her mother up to 16 months after her birth.

Nest groups consisting of three or more animals comprised a mixture of an adult male/female pair and juveniles, which were identified as young from a previous litter. The most frequently observed combination was that of an adult male/female pair and a juvenile female (Table 4.6).

The composition of nest groups appeared to vary according to the reproductive status of the adult female occupying the nest (Figure 4.3). The highest proportion of nests occupied by females suckling back-young (Phase 3 of lactation) contained only the female and her back-young. Occasionally a female was found alone with the back-young in another nest nearby. In contrast, a high percentage of nests occupied by females suckling pouch young (Phase 2a and Phase 2b of lactation) also contained an adult male (Figure 4.3). No lactating females were found in nests occupied by four animals (Figure 4.3).

The frequency with which ringtail possums changed nest sites over a five day period is presented in Table 4.7 Males were found to move from one nest to another

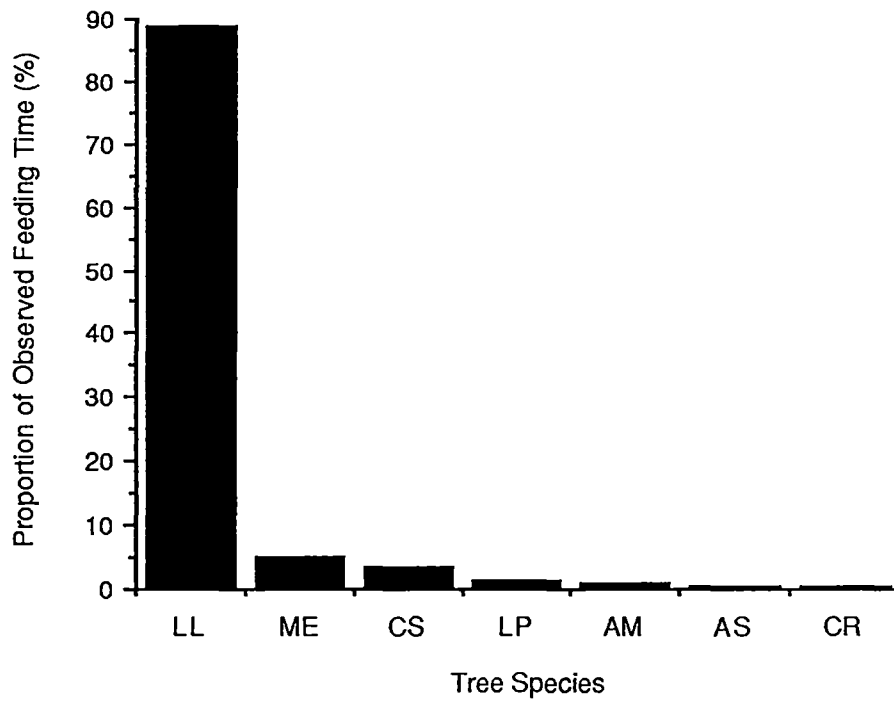


Figure 4.4 The proportion of observed feeding time spent feeding in;
LL = *Leptospermum laevigatum*,
ME = *Melaleuca ericifolia*,
CS = *Casuarina stricta*,
LP = *Leucopogon parviflorus*,
AM = *Acacia mucronata*,
AS = *Acacia sophorae*,
CR = *Callitris rhomboidea*.

more often than females ($df = 45$, $t = 2.52$, $P < 0.05$). There was no significant difference between the number of nest site changes made by non-lactating females and females suckling pouch young ($df = 15$, $t = 1.23$, $P > 0.05$). However, females suckling back-young made significantly fewer changes in nest site than non-lactating females ($df = 14$, $t = 3.70$, $P < 0.01$)

4.3.3 Feeding

Feeding observations were made on fifteen animals during the Spring/Summer months and nineteen animals during the Autumn/Winter. Figure 4.4 shows that foliage from coast teatree, *Leptospermum laevigatum*, constituted the major portion of the diet (89% of observed feeding time). The remainder of the observed feeding time was made up by feeding on *Melaleuca ericifolia*, *Casuarina stricta*, *Leucopogon parviflorus*, *Acacia mucronata*, *A. sophorae* and *Callitris rhomboidea*. Animals were only observed feeding on the last five tree species during the Autumn/Winter months (Table 4.8). In addition, observations of animals feeding in *M. ericifolia* were only made during the Spring/Summer months (Table 4.8). This Spring/Summer feeding in *M. ericifolia* coincided with observations of flowering by this species. However, the dense foliage prevented detailed observations of the particular food component selected by an animal..

4.3.4 Chemical composition of feed leaves

The chemical composition of the tree foliage on which ringtail possums were observed feeding is presented in Table 4.9. Composition determinations were made on winter and spring samples of *Leptospermum laevigatum* and *Melaleuca ericifolia*, but, there was no obvious seasonal difference in composition. Therefore, the composition values presented for these two species are means of the combined winter and spring samples.

All the leaf samples analysed were high in cell-wall content. *Acacia sophorae* had the highest concentration (73% dry matter), whereas *Callitris Rhomboidea* and *L. laevigatum* had the lowest total cell-wall concentration (60.8% dry matter). This lower total cell-wall content for *L. laevigatum* was due to a lower cellulose concentration compared with the other species. The estimates of hemicellulose

Table 4.8 Seasonal comparison of estimated time spent feeding on foliage of different tree species by ringtail possums at Whitemark Beach. Total feeding observation time for each season is given in parentheses.

Tree Species	Proportion of total time observed feeding (%)	
	Spring/Summer (18 hrs 23 mins)	Autumn/Winter (24 hrs 6 mins)
<i>Leptospermum laevigatum</i>	88.03	89.3
<i>Cassuarina stricta</i>	-	5.53
<i>Melaleuca ericifolia</i>	11.97	-
<i>Callitris rhomboidea</i>	-	0.7
<i>Acacia sophorae</i>	-	1.04
<i>Leucopogon parviflorus</i>	-	2.07
<i>Acacia mucronata</i>	-	1.40

Table 4.9 Composition of foliage from tree species eaten by ringtail possums at Whitemark Beach.
 LL = *Leptospermum laevigatum*, ME = *Melaleuca ericifolia*, CS = *Casuarina stricta*,
 AM = *Acacia mucronata*, CR = *Callitris rhomboidea*, AS = *Acacia sophorae*.

Constituent (% dry matter unless otherwise stated)	Tree Species					
	LL	ME	CS	AM	CR	AS
Dry matter (% fresh wt)	49.4	44.9	46.2	39.0	39.3	36.0
Ash	2.8	4.5	5.2	4.2	3.5	2.9
Total Nitrogen (gm N%)	0.9	1.3	1.4	1.5	1.1	-
Crude Protein (Nx6.25)	5.8	7.9	8.9	9.1	6.6	-
Cell wall constituents:						
Neutral-detergent fibre	60.8	68.4	65.1	68.8	60.8	73.6
Hemicellulose	7.3	6.3	14.3	0.8	-	-
Acid-detergent fibre	53.5	62.1	59.8	68.1	-	-
Cellulose	16.6	28.0	27.1	21.6	-	-
Lignin	36.9	34.0	23.7	46.5	-	-
Total Phenolics	6.8	9.2	6.1	7.2	5.8	-
Crude Lipid	7.0	5.0	5.4	4.6	6.0	-
Gross Energy (KJ/g)	23.0	21.3	21.1	21.0	22.3	20.3

Table 4.10 Water content (% fresh wt) of foliage from tree species eaten by ringtail possums at Whitemark Beach. Values are means of duplicate determinations.

Tree species	% Water Content	
	Spring/Summer	Autumn/Winter
<i>Leptospermum laevigatum</i>	50.7	51.1
<i>Melaleuca ericifolia</i>	57.0	52.1
<i>Casuarina stricta</i>	53.8	50.6
<i>Acacia mucronata</i>	56.2	61.5
<i>Acacia sophorae</i>	64.0	62.5
<i>Callitris rhomboidea</i>	55.0	60.7
Mean ± SD	56.1 ± 4.45	56.4 ± 5.7

concentration were highly variable between different species. However, this was probably largely due to its determination as the difference between total cell-walls and acid-detergent fibre.

There was little variation in total phenolic content (ranging from 6.8% to 9.2% of dry matter) between the foliage of different tree species. Similarly, crude lipid content varied little between species *L. laevigatum* had the highest and *A. mucronata* had the lowest crude lipid content (7.0% and 4.6% of dry matter, respectively). Crude protein content ranged from 5.8 % to 9.1 % of dry matter. *L. laevigatum* had the lowest total nitrogen content and *A. mucronata* had the highest (0.93 % and 1.46 % of dry matter, respectively). Gross energy content ranged from 20.3 to 22.7 kJ/g. The highest value of gross energy was measured in *L. laevigatum*.

The water content of foliage from each tree species collected at different times of the year is presented in Table 4.10. There was no significant seasonal difference in the water content of the leaf samples (paired Student's t-test, $df = 5$, $t = -0.18$, $P > 0.05$). Coast tea-tree, *L. laevigatum* had the lowest water content (50.9% fresh weight). The highest water content was determined for *A. sophorae* foliage (63.2% fresh weight).

4.4 Discussion

4.4.1 Activity

The ringtail possums spent most of their time either feeding or resting, which is consistent with the conservative activity budget shared by herbivores in general (Hudson, 1985). A greater proportion of the activity time was spent feeding during the autumn/winter months than during the spring/summer months. Tonkin (1983) found an increase in the length of the active phase of the diurnal red squirrel, *Sciurus vulgaris* as daylength increased during the summer. Similarly, the length of the active phase of the nocturnal ringtail possum may increase during the extended dark phase of the autumn/winter days. Therefore, as well as an increase in the proportion of active time taken by feeding the actual length of time spent feeding by the ringtail possum may be longer at this time of year.

Animals were observed feeding on the less abundant tree species during the

autumn/winter months. This may relate to the quality of the foliage at this time of year. For example, if the quality of the most abundant tree species, *Leptospermum laevigatum* (see Chapter 2) is low during the autumn/winter months a longer period of feeding activity may be necessary at this time of year for successful acquisition of supplementary food resources. Alternatively, higher metabolic demands associated with the lower ambient temperatures during the winter months (Chapter 2) may necessitate prolonged feeding. In addition, increased activity associated with mating behaviour also occurs at this time of year, which must place additional energetic demands on the adult ringtails as also suggested for *Petaurus breviceps* (Goldingay, 1984).

Many studies of mammalian activity have noted a biphasic pattern, with a peak at the onset of activity and a second activity peak prior to the cessation of activity (*Apodemus sylvaticus*, Millar, 1955; *Phascolarctos cinereus*, Robbins and Russell, 1978; *Sciurus vulgaris*, Tonkin, 1983; *Petaurus breviceps*, Goldingay, 1984; *Trichosurus vulpecula*, Winter, 1975 cited in MacLennan, 1984). Thompson and Owen (1964) note that ringtail possums were normally most active in the period prior to midnight. Ringtails observed during this study also appeared to show a peak of activity during the first half of the night. This was followed by a rest period and then a second activity peak before they returned to the nest which indicates that the ringtail possum also exhibits a biphasic activity pattern.

The mechanism of this biphasic activity pattern is not understood (Tonkin, 1983). However, since the pattern is usually characterised by two feeding periods divided by a rest period, many authors have suggested its function is related to food processing (Tonkin, 1983; Goldingay, 1984). The break between the early feeding period may become necessary when the stomach reaches its capacity. Extensive periods of inactivity following feeding bouts have been noted in many herbivorous mammal species (Herbers, 1981; *Trichosurus vulpecula*, MacLennan, 1984; *Spermophilus saturatus*, Kenagy, 1987; *Marmota flaviventris*, Melcher *et al.*, 1989). In these studies the rate of intake of the bulky fibrous diet depends on the rate of passage of material through the gut. The latter is usually slow in herbivores because of the low digestibility of their fibrous diet (McDonald *et al.*, 1988). Kenagy (1987) proposed that the herbivore *S. saturatus* rested while its gut was full and actively digesting.

More specifically, Cork and Warner (1983) have suggested that the intake of

foliage by arboreal folivorous marsupials may be limited by the 'gut-filling' effect of the leaf cell-wall constituents. Digesta retention times recorded for folivorous marsupials, including the ringtail possum (Chilcott and Hume, 1985) are slow compared with those of many herbivorous domestic species (Van Soest, 1982). The large proportion of time spent feeding by the ringtails was accompanied by a large proportion of time spent 'stationary'. Similar long periods of inactivity have been recorded for other marsupial folivores (i.e *Phascolarctos cinereus*, Smith, 1979b; *Petauroides volans*, Kehl and Borsboom, 1984; *Trichosurus vulpecula*, MacLennan, 1984). Therefore, the ringtail possum and other folivorous marsupials probably remain stationary between feeding bouts whilst digesting their meal of leaves.

In addition to the digestibility-reducing fibre, leaves also contain non-nutritional compounds such as essential oils, tannins and phenols (Feeny, 1970; Cork *et al.*, 1983; Cork and Pahl, 1984) which may affect folivores physiologically as metabolic toxins or by disrupting the digestive process (Freeland and Janzen 1974, Cork and Sanson 1989, see Chapter 1). Digestion of these 'allelochemicals' or 'secondary compounds' involves detoxification and excretion. Detoxification is regarded as an energetically expensive process (Hinks and Bolliger, 1957; Freeland and Janzen, 1974). However, Cork and Sanson (1989) propose that the major metabolic consequence of allelochemicals in *Eucalyptus* leaves is the proportion of unusable energy that they represent in the leaf. Therefore, the ringtail possum is probably energy limited and the large proportion of time spent stationary may be a behavioural strategy to minimise energy expended in activity. This would assist in maintaining an adequate energy balance. A similar scenario has been proposed for *T.vulpecula*, *P. cinereus* and *P.volans* (MacLennan, 1984) and is consistent with the hypothesis that arboreal folivores, in general, conserve energy by minimising activity costs (Eisenberg, 1978; McNab, 1978).

Although the ringtail possum shares 'inactivity' with other marsupial folivores (*P.cinereus*, Smith, 1979b; *P. volans*, Kehl and Borsboom, 1984; *T.vulpecula*, MacLennan, 1984) the proportion of 'stationary' time varies among species. Sitting made up 40 to 50% of total activity time for *T. vulpecula* (MacLennan, 1984) and *P.volans* (Kehl and Borsboom, 1984) whereas in ringtails it represents approximately 28% of observed activity time. The ringtail possum also spends a greater proportion of time feeding than these other folivorous species. The

reasons for these differences are not obvious. However, they may be related to the shorter digesta retention times of ringtail possums compared with those of other marsupial folivores (Hume *et al.*, 1984; Chilcott and Hume, 1985). In addition, lower levels of total phenolics in the leaves eaten by ringtails in this study compared with *Eucalyptus* leaves, on which the other species were feeding, may have enabled a higher rate of food intake.

Occasionally, while the ringtails were sitting in one position, they would emit a 'chirruping' call (see Appendix B) and frequently this call was answered by other possums in the vicinity. 'Stationary' periods may therefore also serve some social function, such as exclusion of intruders from a home range, predator surveillance (Melcher *et al.*, 1989), or maintenance of contact between family groups whilst feeding.

Males spent more time feeding and less time moving than females. This could be related to a larger home range area for adult females. However, Pahl (1985) found that male ringtails in Victoria had larger and more exclusive home ranges than the females. Miscellaneous activities (such as returning to the nest during the active period) also involve travelling from one position to another. Therefore, since these activities were performed more by females than males they would have contributed to the greater proportion of time spent moving by females. However the observed greater proportion of time spent feeding by males compared with females remains to be explained. Since there is no sexual dimorphism in adult ringtail possums this observation cannot be related to a difference in energy requirements of the sexes. Most of the observations of male ringtail possums were conducted during the autumn/winter months, therefore, the comparison with females may be biased by seasonal influences on the amount of time spent feeding by males.

The major difference between females of different reproductive status was in feeding, with females suckling back-young (Phase 3 of lactation) spending 14% more of their active time (approximately 1 hour more actual time) feeding than non-lactating females. Increased length of feeding or foraging bouts has also been noted for lactating females in studies of other mammal species (*Tamasciurus* sp, Smith, 1968; *Caluromys philander*, Atramentowicz, 1982; *Papio cynocephalus*, Altmann, 1983; *Macropus rufogriseus rufogriseus*, Clark and Loudon, 1985; *Marmota flaviventris*, Melcher *et al.*, 1989). Such extensions of feeding time may be associated with the increased energy requirements of lactating females (see Chapter 6).

It was suggested earlier that the 'stationary' periods observed for ringtail possums between feeding bouts were a result of a limit to the processing capacity of the alimentary tract caused by the 'gut-filling' effect of fibre. Therefore the observation that late lactation females spent more time feeding but less time 'stationary' than the other groups of females was surprising. However, females

were in the late stages of lactation during the spring and summer months (Chapter 2) when young foliage is most abundant (Pahl 1985; Pahl 1987b). The young foliage of some tree species has been found to contain less fibre than mature foliage (Oates *et al.*, 1980; Baranga, 1983; Cork and Pahl, 1984) and Pahl (1985, 1987b) has shown that ringtail possums prefer to consume young foliage when it is available. Therefore, if females suckling back-young were feeding on young foliage and assuming this foliage had a lower fibre content than mature foliage, a faster rate of digesta passage would be expected.

Surface area determines heat loss so that small animals lose more heat per unit body weight than large animals and must therefore eat proportionally more in order to support the increased heat production necessary to compensate for the heat loss. Many species compensate to some degree for the deficiencies of their own insulation by creating their own microclimate (i.e., burrowing, nest-building). Both groups of lactating females (Phase 2 and Phase 3 of lactation) spent a greater proportion of time in the nest during the night than non-lactating females. Females are usually carrying pouch young (Phase 2 of lactation) during the winter and early spring months when minimum ambient temperatures are up to 15°C below the thermoneutral zone of the ringtail possum (see Chapter 2). Therefore returning to the nest at periods during the night may be a strategy adopted to minimise thermoregulatory energy costs at this time of year. Late lactation generally occurred during late spring and summer (see Chapter 3) when the minimum ambient temperatures were rarely less than 10°C below the thermoneutral zone of *P. peregrinus viverrinus* (see Chapter 2). Therefore, the thermoregulatory costs for females suckling back-young would be expected to be low and the time spent in the nest during the night at this stage of lactation is less likely to be a strategy to conserve energy. An alternative explanation could be that these females return to the nest to suckle their young.

4.4.2 Nest Occupancy

The nest groups of the ringtail possum in this study appeared to be composed of members of a particular family group as described in studies of other ringtail populations (Thompson and Owen, 1964; Marsh, 1967; How *et al.*, 1984). Intolerance between males may be the reason for the relatively few instances in

which two or more adult males shared the same nest. Although immature animals of a previous litter were occasionally found sharing a nest with an adult male and female pair, juvenile males were rarely found in a nest with their parents after they had reached sexual maturity. This suggests that adult males, as well as being intolerant of unrelated males, are intolerant of their male offspring. In contrast, daughters were often found sharing a nest with their parents after they had become sexually mature. Henry (1984) has proposed that elimination of breeding competition is the basis of the intolerance of juvenile males by adult male *P. volans*. Similarly Pahl (1985) proposed that older dominant male *P. peregrinus* exclude the young males from females.

The long term fate of young male ringtails was not investigated in this study. However, a similar intolerance of male offspring noted for *T. vulpecula* (Dunnet, 1964) and *P. volans* (Henry, 1984) is followed by dispersal of juvenile males leading to a female-biased sex ratio. Nevertheless, Pahl (1985) did not find a pattern of male biased dispersal in *P. peregrinus*. In addition, the sex ratio of populations of *P. peregrinus* in Victoria (Thompson and Owen, 1964; Hird, 1975) and in this study did not appear to differ significantly from the 1:1 ratio. How *et al.* (1984) did find the sex ratio to be biased toward females in a population at Sandy Point, Victoria but they attributed this to the higher survival of adult females compared with adult males (also noted by Pahl, 1987a) and the longevity of the possums in this population. Further studies on the survival, dispersion and sociality of *P. peregrinus* are needed before the fate of young male ringtails excluded from the family group can be resolved.

During the breeding season and when the females are suckling pouch young (April-September, see Chapter 3) male and female pairs were common. Furthermore, nests containing more than two adults or juveniles were mainly encountered at this time. The larger nest groups could be a result of the social behaviour of ringtails at this time of year. However, there may also be a physiological advantage gained by the larger sleeping groups. Smith and Lee (1984) suggest that the formation of sleeping groups noted in the smaller species of arboreal marsupials may be related to heat conservation. Individual ringtail possums were found on their own most commonly during the spring and summer months, both in this study and by How *et al.* (1984). The larger nest groups during the winter months may therefore be a strategy to minimise energy costs associated with

thermoregulation during the cooler winter days.

Data presented here and by Thompson and Owen (1964) suggest that nest sharing between mated adults of *P. peregrinus* tends to cease after the young have emerged from the pouch. This was similar to the situation observed in *P. volans* by Henry (1984) in which nest sharing only involved females who had failed to raise young. Smith and Lee (1984) propose that, when solitary, females need not share a resource and hence may obtain more nutrients for reproduction. Alternatively, the tendency of female ringtails to remain alone whilst suckling back-young may have a more specific physiological basis. Ringtail possums appear to suffer heat stress during hot summer days (Pahl, 1987a; personal observation): to facilitate evaporative heat loss they lick their paws, forearms and tail. Females suckling back-young would need to avoid heat stress at a time when their energy and water requirements are greatest (see Chapter 6). Therefore exclusion of males from the maternal nest may be a strategy to minimise the sleeping group size and hence reduce the chance of heat stress.

Females remain in a nest site after the young have emerged from the pouch. Similar observations were made by Morton (1976, cited in Read, 1985) who states that the presence of pouch young did not deter female *Sminthopsis crassicaudata* from moving nest sites, but females with older young were most likely to be caught at the same nest site. In some mammal species, particular nests are built for protection of the young from predators and warmth. These maternal nests appear to be larger and more dense than ordinary sleeping nests (Walser, 1977). Several observations were made in this study of ringtail possums moving on the ground. Attempts to observe the animals behaviour on the ground were not successful since they were easily disturbed. However, most of these observations were made during mid-late spring and coincided with the appearance of new nests in the study area during October 1987 prior to emergence of the young ringtail possums from the pouch. Thompson and Owen (1964) also note increased nest-building activity at a time when most of the female ringtail possums carried pouch-young. Perhaps the construction of particular maternal nests is the reason behind this increased nest building activity.

4.4.3 Diet and Food Composition

In this study direct observation of ringtail possums feeding at Whitemark

Beach suggested that their diet consisted primarily of *Leptospermum laevigatum* foliage supplemented by foliage from the less abundant tree species in the study area. Observer disturbance may limit the effectiveness of direct observation as a means of quantitatively determining diet composition (Pahl, 1987b). In addition, the proportion of time spent feeding may not represent actual intake of a particular species since particular parts of the tree may require different handling times. For example, young leaves may have a lower fibre content than more mature leaves (Cork and Pahl, 1984), hence less time would be needed for mastication and digestion. However, analysis of faecal pellets collected from ringtail possums inhabiting a coast teatree thicket at Sandy Point in Victoria has shown that the diet of *P. peregrinus* there consists almost entirely of *L. laevigatum* foliage (Pahl, 1987b).

The feeding observations of ringtail possums at Whitemark Beach suggested that the second most abundant tree species, *Melaleuca ericifolia*, formed part of the diet during the spring and summer months but not during the winter. *Melaleuca ericifolia* produces flowers during the spring and early summer months at Whitemark Beach and since ringtail possums are known to consume flowers of other species (Pahl, 1987b), consumption of *M. ericifolia* flowers may account for the observations made of possums feeding in this tree species.

Pahl (1987b) showed that the proportion of young *L. laevigatum* foliage in the faeces of ringtails at Sandy Point was highest during the spring and summer. This coincided with an increase in production of young *L. laevigatum* foliage (Pahl 1987b). Assuming that *L. laevigatum* shows a similar pattern of young leaf production at Whitemark Beach, these potentially more nutritious leaves would be less abundant during the autumn and winter months. Therefore, the observations of ringtails feeding in the less abundant tree species during the winter may reflect a need to supplement the *L. laevigatum* component of the diet. However, more information is needed on the phenology of the tree species in the study area and the chemical composition of the leaves at different stages of growth.

Proximate analysis of the leaves from the tree species in which ringtails were observed feeding revealed a high concentration of total cell-walls and lignin (i.e., 60.8-73.6% and 24-46% dry matter respectively). These values are greater than the concentrations of cell-walls and lignin found in eucalypt species (23 - 56% and 9 - 27% respectively, reviewed in Cork, 1984; Foley, 1987) which constitute the major

food source for ringtail possums in other areas (Thompson and Owen, 1964; Pahl, 1984, '85, '87b). However, these high levels of fibre were similar to those found in understory tree species (i.e., *Leptospermum juniperum*, *Acacia melanoxylon*) of eucalypt forest (Cork and Pahl, 1984).

Although the fibre content of the foliage on which the ringtails were observed to feed at Whitemark Beach was particularly high, the concentration of other digestibility-reducing compounds (i.e., total phenols) appeared to be low. In general, total phenolic concentrations were lower than those recorded for eucalypt species and understory tree species (Cork, 1984; Cork and Pahl, 1984). However, in this study, leaf samples were analysed for total phenolics after they had been dried and since drying may cause some oxidation of phenolic compounds this may have lead to underestimates (S.Cork, personal communication). Nevertheless, Cork (unpublished data) found similar low levels of total phenolics in samples of *L. laevigatum* foliage which were analysed without prior drying. He also noted correspondingly low levels of tannins.

Levels of crude lipid were also relatively low when compared with the available information on lipid concentration in eucalypt foliage (reviewed in Cork, 1984; Foley, 1987). It is postulated that this difference is due to a lower concentration of essential oils in the foliage analysed in this study. However, total nitrogen contents of the leaves analysed were within the range of values available for other tree species which the ringtail possum is known to consume (Cork, 1984b; Cork and Pahl, 1984).

In summary, the foliage available as food for ringtail possums at Whitemark Beach shares the low nutritional qualities of foliage eaten by ringtail possums and other marsupial folivores in other areas. As suggested for folivores in general (McNab, 1978; McNab, 1980), this low quality diet may result in a low rate of energy intake by the ringtail possum. Observations made in this study of the behaviour and activity patterns of the ringtail possum are consistent with the suggestion that arboreal folivores conserve energy by minimising activity costs more than other mammals (Eisenberg, 1978; McNab, 1978). The time spent on different activities by females varied according to their reproductive state which may reflect the strategy employed to cope with increased energy and nutrient requirements for lactation.

CHAPTER 5

STANDARD METABOLISM, DIGESTION, AND VALIDATION OF THE USE OF TRITIATED WATER FOR ESTIMATING WATER INTAKE IN THE COMMON RINGTAIL POSSUM.

5.1 Introduction

The combination of the low energy availability of foliage diets and a small body size dictated by arboreality has led to the suggestion that arboreal folivores conserve energy more than other mammals (see Chapter 1). McNab (1978) concluded that a low basal metabolic rate (metabolic rate of rested, awake and fasted animals at thermoneutrality; Bligh and Johnson, 1973) is a characteristic shared by arboreal folivores regardless of taxonomic group. However, more recent studies have shown that although some arboreal folivores exhibit a low basal metabolic rate during the normal period of inactivity compared with that of other mammals in the same taxonomic group (i.e., *Phascolarctos cinereus*, Degabriele and Dawson, 1979; *Bradypus variegatus*, McNab, 1978) others do not (i.e., *Petauroides volans*, Foley, 1987).

Few studies have been made on the metabolism of the common ringtail possum. However, Kinnear and Shield (1975) reported a basal metabolic rate value of $2.54 \text{ W.kg}^{-0.75}$ ($219.5 \text{ kJ.kg}^{-0.75}.\text{day}^{-1}$) in the western ringtail possum, *Pseudocheirus peregrinus occidentalis*, which is close to the value predicted, for a marsupial of this body mass, from Dawson and Hulbert (1970). Chilcott and Hume (1984a) investigated the digestion and metabolism of *Eucalyptus andrewsii* foliage by the common ringtail possum and found that the digestible energy required for maintenance (e.g., cost of thermoregulation, minimal activity etc.) was $436 \text{ kJ.kg}^{-0.75}.\text{day}^{-1}$. This is 1.9 times the basal metabolic rate value estimated for *Pseudocheirus peregrinus occidentalis* (Kinnear and Sheild, 1975). The maintenance energy requirements of other herbivorous marsupials are also approximately double their basal metabolic rate (Hume, 1982).

Estimates of an animals metabolism, made in the laboratory, can only be applied to the field with caution since free-living animals are responding to

environmental variations (i.e., food supply, food quality, weather, reproductive status etc.). Nagy (1987) compared the allometric relationship between the respiratory energy metabolism of an animal in free existence (field metabolic rate) and body mass for a number of eutherian mammals and marsupials and found that field metabolic rate scales differently from basal metabolism.

Field metabolic rate estimates include the total energy costs of free existence (e.g., thermoregulation, activity, growth, reproduction) and can be measured indirectly using doubly labelled water (Lifson and McClintock, 1966). This method involves enriching an animal's body pool water with isotopes of hydrogen and oxygen (i.e., ^3H or ^2H and ^{18}O). The differential washout rates of these isotopes provide measures of water gain, water loss and CO_2 production (respiratory energy metabolism) of an animal (Nagy, 1989a). Validation studies on the use of isotope turnover techniques in birds and mammals (e.g., Nagy, 1980; Nagy and Costa, 1980; Gales, 1989) highlight the errors that may occur and the importance of estimating the magnitude of such errors before interpreting results obtained in studies of free-living energetics.

The food requirements of an animal can also be estimated using the doubly labelled water technique if the diet composition and digestibility are known (Nagy, 1989a). A feeding trial was conducted in this study to obtain estimates of metabolisable efficiencies which subsequently enabled conversion of the field metabolic rate measurements, made in the following chapter, to quantitative estimates of food intake. In addition, the use of the tritium isotope (^3H) to obtain an estimate of water influx was validated by comparison with direct measurement of water influx.

Standard metabolic rate (basal metabolic rate is a particular case of standard metabolic rate, Bligh and Johnson, 1973) is the most commonly used comparative energy unit (e.g., Dawson and Hulbert, 1970; McNab, 1980; McNab, 1986a) and the field energy expenditures of individuals are often expressed as multiples of standard metabolic rate to facilitate comparisons between species (e.g., Smith *et al.*, 1982; Hume *et al.*, 1984; Nagy and Martin, 1985; Nagy and Suckling, 1985). Therefore, standard metabolic rate of *Pseudocheirus peregrinus viverrinus* was measured in the present study to enable comparison of the ringtail possums' field metabolic rate with that of other species (see Chapter 6).

5.2 Methods

5.2.1 Measurement of Standard Metabolic Rate

Animals

Five healthy adult female ringtail possums maintained in captivity (see Chapter 2) were used in this study. Initially the possums were deprived of food overnight prior to an experiment the following day. However, due to a relatively large drop in the body mass, overnight, this procedure was discontinued. It was not feasible to remove the food provided during the night, therefore food was provided in small quantities and measurements were made between 13:00 and 16:00 hrs the following day.

Measurements

Metabolic rates were measured with a positive pressure open circuit system previously described by Nicol (1976). The metabolic chamber consisted of an insulated 251 double-walled fibreglass container. Temperature within the chamber could be regulated to within 0.1 °C by controlling the temperature of brine circulating through the outer annulus, and a heater/cooler unit. Dry and wet bulb temperatures were recorded continuously by thermocouples secured within the chamber. Air flow rates through the chamber (2.632 l.min^{-1} STP) were measured with a calibrated Gilmont flowmeter.

Outlet chamber air passed through a 50 cm³ cylinder of desiccant (Drierite) to the sample channel of a Taylor Servomex OA 184 oxygen analyser and a Beckman LB-2 Medical Gas Analyser. Air pressure inside the chamber as shown by a water manometer was maintained at 9-10 cm water to avoid dilution of the system from atmospheric air. Atmospheric air from an alternative flowmeter simultaneously passed directly through a 600cm³ cylinder of silica gel to the reference channel of the oxygen analyser. Outputs from the sample and reference channels were compared by a Servomex RB-228 ratio box and recorded on a Rikadenki B341 recording potentiometer with a full-scale deflection from 20 to 21% O₂.

Deep body temperature was recorded throughout each experiment by a thermocouple sheathed in plastic and inserted 5 to 6 cm into the cloaca and taped around the base of the tail to prevent loss of the thermocouple during the

experiment. Thermocouple outputs were displayed on a Leeds and Northrup Speedomax 250 recorder. Thermocouples were calibrated against a standard thermometer in a constant-temperature bath to ± 0.1 °C.

At least one hour was allowed for the possum to equilibrate to the chamber temperature and at least one hour for the measurement. All measurements represent steady-state conditions. Determinations of oxygen consumption were made from the lowest levels of the chart recording after it had been stable for at least fifteen minutes; this period corresponded to the possum resting quietly in the chamber with a stable body temperature.

Oxygen consumption and body temperature were measured simultaneously at a range of ambient temperatures. To give a value of standard metabolic rate (SMR) as defined by Bligh and Johnson (1973) each oxygen consumption measurement ($\text{VO}_2 \text{ ml.g}^{-1}.\text{hr}^{-1}$) was converted to Watts. The mean standard metabolic rate is also expressed as $\text{kJ.kg}^{-0.75}\text{d}^{-1}$ and kJ/day to enable comparison with measurements made during the feeding trial and measurements of field metabolic rate (see Chapter 6). Conversion factors (e.g., 1 Watt = 3.6 kJ/hr) were obtained from Kleiber (1961).

5.2.2 Feeding Trial and Validation of Water Influx Rates.

Animals and Experimental Procedure

Three adult non-lactating female ringtail possums were removed from their holding cages (Chapter 2) and placed in individual metabolism cages. The cages were housed in a quiet controlled temperature room ($20^\circ\text{C} \pm 1.0$ °C), which was close to the thermoneutral zone of this species. (see Results). The room was under a 12:12 light:dark regime. Each cage was designed to allow the collection of faeces and equipped with a resting log, water container and wooden nest box with a mesh floor to allow for collection of faeces voided in the nest. Attempts to collect urine were unsuccessful for several reasons. Firstly, contamination of the urine samples was caused by some faecal pellets and leaves which dropped through the mesh floor of the cage into a flask designed for the collection of urine. Secondly, urine tended to accumulate on the resting log and on uneaten leaves which lay on the mesh floor of the cage resulting in underestimates of urine volume.

The animals were maintained in the cages for three days before commencement of the trial to allow them to become accustomed to their new

environment. After this period the animals had maintained mass and their food intake was stable. Attempts were made to locate a local source of the major component of the field diet, *Leptospermum laevigatum*, for use in the feeding trial. However, this species is confined to the North of Tasmania and collection of *Leptospermum laevigatum* foliage for use in the trial (conducted in the South of Tasmania) was not feasible. Therefore, the feed offered throughout the trial consisted of sapling foliage from *Eucalyptus amygdalina*, the major component of the diet of ringtail possums maintained in captivity (Chapter 2). Fresh leaves were collected daily from the same area of Mt. Nelson in Hobart. The ringtail possums held in captivity were observed to feed only during the night, emerging from the nest box at the onset of darkness. Therefore, the collection and feeding protocol was conducted between 1400h and 1700hrs.

Small branchlets of *Eucalyptus amygdalina* were divided into five lots. Each lot was then weighed to within 0.01g on a Mettler PE 3600 balance. One lot was placed in each cage with the stems placed in water outside the cage to maintain freshness. Another lot was placed in water near the metabolism cages and used to estimate the gain or loss in water over the 24hr experimental period. The final lot was stripped of the leaves and these were then divided into two random grab samples for dry matter determination and chemical analyses. A known mass of water was provided in plastic containers secured to the side of each cage and evaporative water loss was estimated from a control water container placed near to the cages.

Each day the voided faeces were collected from each cage, weighed, and stored in sealed plastic bags at -20°C until analysis. Faeces from each animal collected over the whole 6 day measurement period were bulked. The water remaining in the plastic container placed in the cage was weighed after each 24 hr period. Leaves remaining on the branchlets in each cage were collected daily and weighed. The leaves that lay on the cage floor were also collected but weighed separately.

On the first day of the 6 day measurement period each animal was weighed on a Mettler PE 3600 balance to the nearest 0.1g. An initial blood sample was collected by nicking a peripheral ear vein with a scalpel blade. Between 0.5ml and 2ml of blood was then collected into unheparinized plastic vials (Microtainer) for measurement of isotope background levels. The possums were then injected

intraperitoneally with 0.2ml H_2^{18}O (95% + atom excess ^{18}O) and 0.5ml tritiated water (HTO:185 MBq). The injection volumes were calibrated by weighing an equivalent volume of distilled water (assuming 1.0ml = 1.0 g for distilled water) and was found to be accurate to within $\pm 0.01\text{ml}$.

The time required for isotope equilibration may vary with body mass, route of injection and metabolic rate (Kunz and Nagy, 1988). It is important to estimate the period of time for an isotope to equilibrate with the body water pool, as sampling before complete equilibration may underestimate or overestimate total body water and hence produce errors in the subsequent calculation of turnover rates (Gales, 1989; Kunz and Nagy, 1988). Similarly, sampling after the isotopes have reached equilibration will lead to an overestimate of total body water (Kunz and Nagy, 1988). To determine the time required for isotope equilibration, an individual female ringtail (held in captivity) was injected with 1ml tritiated water (HTO: 185 MBq). Serial blood samples were taken at regular intervals following injection. Isotope concentrations at 60, 150 and 195 minutes gave TBW values of 69.7, 71.0 and 70.5% respectively. Therefore animals in this study were bled for equilibration blood samples (approximately 0.5ml) 3hrs after isotope injection. The feeding trial ran for six days and on the last day a final blood sample was collected from each animal for measurement of isotope turnover.

Analytical Procedures

The daily grab samples of feed leaves were dried at 80°C for 24hrs (or to a constant mass) for determination of their dry matter content. A separate dry matter determination was made for uneaten leaves collected from the floor of the cage because they were usually much drier than those left on the branchlets or had been contaminated with urine. Daily dry matter intakes were calculated after correcting for the daily mass change of the leaves due to water gain or loss. The dry matter content of bulked faeces was determined by drying them at 80°C to a constant mass (24 to 48 hrs).

Samples of feed leaves and faeces used for composition analysis and determination of energy content were dried at 50°C for 24hrs after which they were ground in a Wiley mill and stored in sealed plastic bags. The organic matter, total nitrogen, crude lipid, total phenolic, structural carbohydrate and gross energy contents of feed leaves and faeces were determined as described for field leaf

samples in Chapter 4. Hemicellulose content was assumed to be the difference between neutral-detergent fibre (NDF), and acid-detergent fibre (ADF) estimates (Chilcott and Hume, 1984a). Residual (available) carbohydrates were assumed to make up the remaining organic matter i.e., organic matter - (crude protein + total phenolics + crude lipid + structural carbohydrates).

The apparent dry matter digestibility (DMD) and apparent digestibility of gross energy intake (DE) were calculated with the following equations (Grodzinski *et al.*, 1975):

$$\text{DMD} = \frac{\text{dry matter intake (DMI)} - \text{faecal dry mass}}{\text{DMI}} \times 100$$

$$\text{DE} = \frac{\text{gross energy intake (GEI)} - \text{faecal energy output}}{\text{GEI}} \times 100$$

Apparent digestibilities of the feed leaf constituents were calculated from DMD estimates along with dry mass specific values for each constituent in the food and faeces. To facilitate comparison of data between species metabolic parameters have been expressed in terms of metabolic body mass (i.e., $\text{kg}^{0.75}$, Kleiber, 1961).

Frozen blood samples were thawed and water was extracted from the whole blood by vacuum sublimation (Vaughan and Boling, 1961). Where only small blood samples had been obtained (i.e., < 0.5ml) water was extracted by heat distillation. Aliquots of each extracted water sample (20 μl) were then pipetted into 3ml of PCS cocktail (Phase Combining System, Amersham) in a scintillation vial using a self levelling capillary tube (Drummond). The extracted water was then assayed (in duplicate) for tritiated water activity in a liquid scintillation spectrophotometer (Beckman LS 2800).

The concentration of oxygen-18 in water extraction was also measured, however a malfunction in the isotope ratio mass spectrometer resulted in unreliable ^{18}O values for these samples, particularly for the animal blanks. Consequently this experiment could not be used as a validation trial between doubly-labelled water and materials balance methodology.

A standard dilution of the tritiated water injection solution was prepared. (i.e., 0.5 ml of tritiated water was made up to 1000 ml with distilled water) and analysed for tritium isotope activity as described above. Body water pool sizes

(TBW) were calculated by comparing blood isotope levels at equilibration to standard dilutions of the injected tritium isotope.

$$\text{i.e., TBW}_1 (\text{ml}) = \frac{\text{St} - \text{Bk}}{\text{count}_1 - \text{Bk}}$$

$$\% \text{BW} = \frac{\text{TBW}_1 (\text{ml}) \times 100}{\text{M}_1 (\text{g})}$$

$$\text{TBW}_2 (\text{ml}) = \frac{\% \text{BW} \times \text{M}_2 (\text{g})}{100}$$

where;

St = isotope standard counts (DPM)

Bk = background counts (DPM)

Count₁ = equilibration count (DPM)

M₁ = mass of animal at equilibration (g)

M₂ = mass of animal at recapture (g)

Rates of water influx and water efflux were calculated from isotope activities using the equations of Lifson and McClintock (1966, as equation 4), Nagy and Costa (1980, as equation 3). Body water volumes at recapture were assumed to have the same proportion of body mass as at injection. It was assumed that changes in body mass reflect changes in pool sizes and that any changes in these parameters were linear during the experimental period. Any change in body mass was estimated as a percentage of the animals mean body mass for the duration of the measurement period.

5.2.3 Statistical methods

All means are presented \pm 1 standard deviation.

5.3 Results

5.3.1 Standard Metabolic Rate, Body Temperature and Thermoneutral Zone.

The changes in respiratory energy metabolism (watts) and colonic temperature with changing ambient temperatures in the five adult female ringtail possums are shown in Figure 5.1a and Figure 5.1b, respectively. The thermoneutral zone as defined by Bligh and Johnson (1973), and delineated by eye for each animal, ranged between ambient

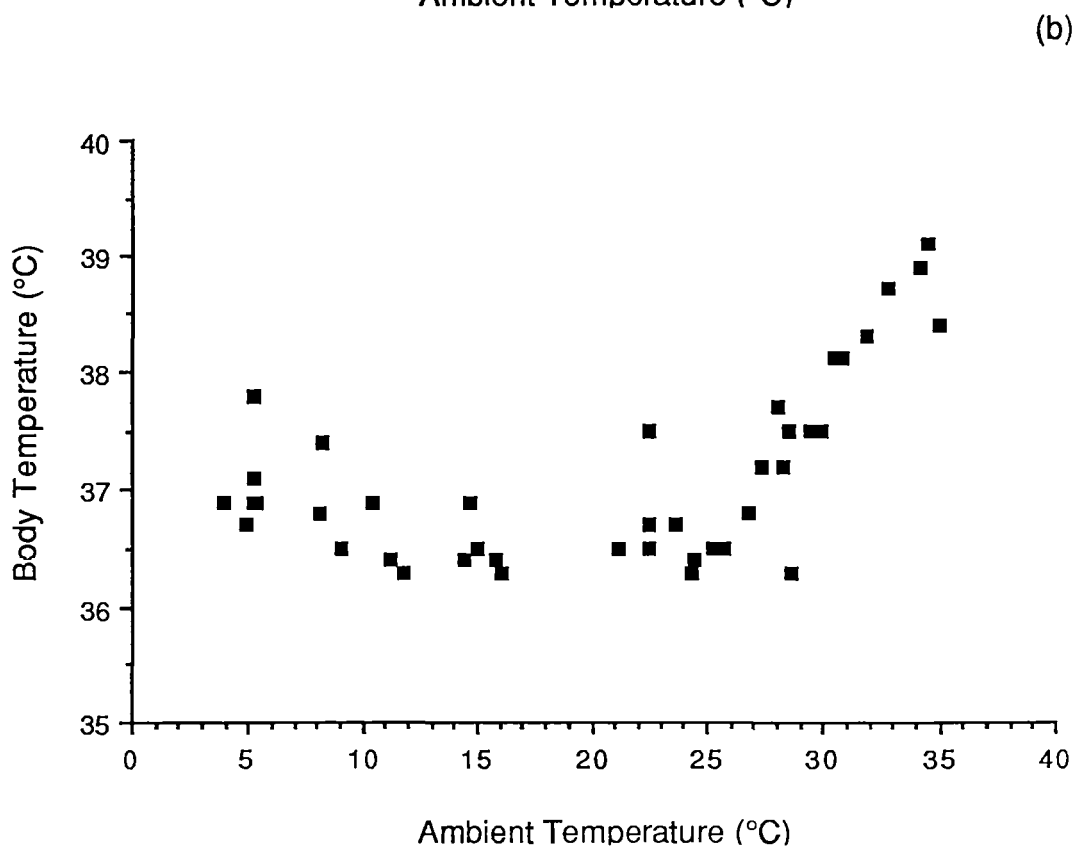
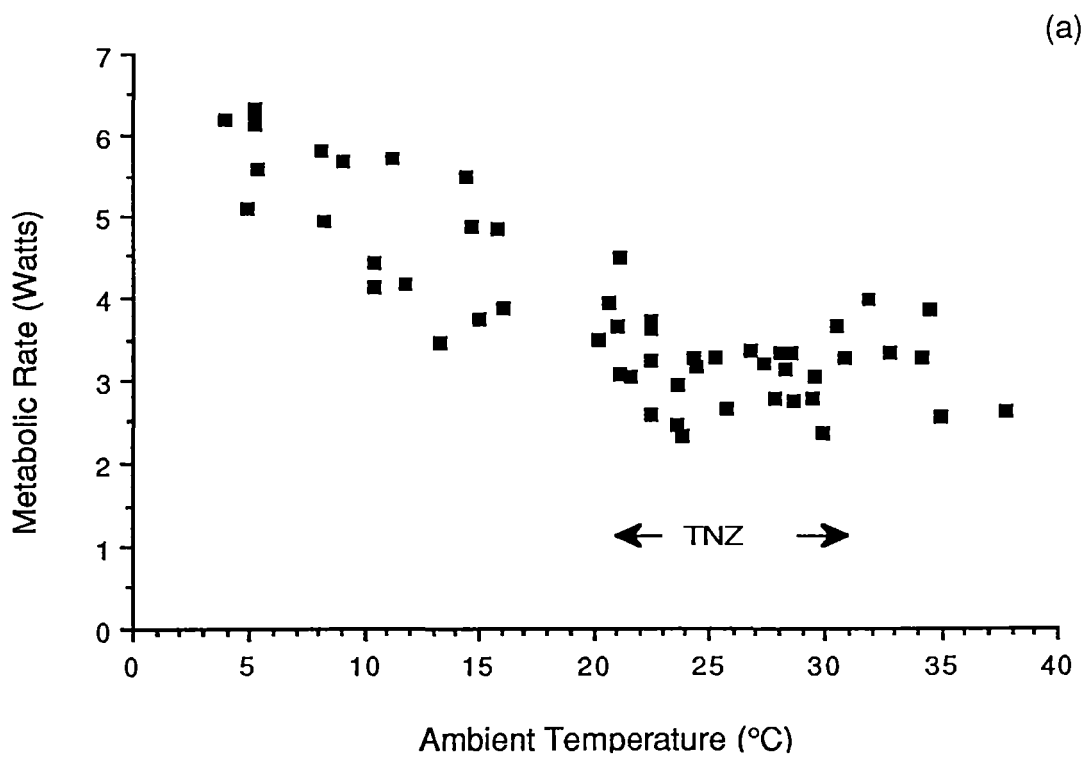


Figure 5.1 The effect of ambient temperature on, (a) the metabolic rate (watts) and (b) the colonic temperature (°C) of *Pseudocheirus peregrinus viverrinus*.

temperatures of 20°C-21°C and 30°C-31°C (Figure 5.1a). The mean metabolic rate of the possums within this thermoneutral zone (standard metabolic rate) was 2.80 ± 0.2 watts, $n=20$ with an RQ of 0.86 ± 0.08 (mean body mass = 890 ± 69 g). This mean estimate of standard metabolic rate is equivalent to 241.9 kJ.d^{-1} or $265.8 \text{ kJ.kg}^{-0.75}\text{d}^{-1}$.

Minimum colonic temperature was $36.9 \pm 0.6^\circ\text{C}$ ($n=20$). Homeothermy was maintained well in the cold with the possums exhibiting changes in body posture as the ambient temperature decreased below 15°C (i.e., curling into a tight ball with all extremities hidden and the tail wrapped around the head). The elevation of body temperature above the minimum value was more pronounced at high ambient temperatures than at low ambient temperatures. At ambient temperatures above 31°C the possums exhibited changes in body posture which presumably facilitated evaporative cooling i.e., the tail was extended and coated with saliva. As the ambient temperature was increased toward 40°C the possums lay on their backs with all extremities extended and coated in saliva.

5.3.2 Feeding Trial

Feed Composition

The composition of the *Eucalyptus amygdalina* foliage used in the digestibility trial is given in Table 5.1. The fibre content (NDF) of *E. amygdalina* foliage made up approximately 50% of the leaf dry matter. Total nitrogen made up less than 2% of the leaf dry matter. Crude lipid and total phenolics made up 5.33 % and 7.30 % of leaf dry matter, respectively. The gross energy content was 20 kJ.g^{-1} dry matter.

Body Mass, Food Intake and Digestibility of Dry Matter and Energy

The body masses, dry matter intakes, apparent dry matter digestibilities, energy intake and energy digestibilities for the three animals in the feeding trial are given in Table 5.2. Body mass changes for the individual ringtail possums in the feeding trial were less than $\pm 5\%$ of the initial body weight. This indicates that the animals maintained condition throughout the trial. Mean dry matter intake was $46.58 \pm 7.67 \text{ g.kg}^{-1}.\text{d}^{-1}$ ($n=3$). The apparent digestibility of gross energy was $47.96 \pm 3.21 \%$ ($n=3$), resulting in a mean digestible energy intake of 465.85 ± 73.63

Table 5.1 Composition of *Eucalyptus amygdalina* foliage.

Constituent (% dry matter unless otherwise stated)	
Dry matter (% wet weight)	43.83
Organic matter	97.10
Ash	2.90
Total nitrogen (N)	1.21
(A) Crude protein (N x 6.25)	7.54
(B) Total phenolics	7.30
(C) Crude lipid	5.33
Structural Carbohydrates	
(D) Neutral detergent fibre	49.98
(E) Acid detergent fibre	48.07
Cellulose (E-F)	19.44
(F) Lignin	28.58
Hemicellulose (D-E)	1.91
Non-Structural Carbohydrate (Organic matter - A+B+C+D)	26.95
Gross Energy (kJ.g ⁻¹ dry matter)	20.18

Table 5.2 Body mass, food intake and digestibility of dry matter and energy by three ringtail possums.

	1	Animal 2	3	Mean \pm s.d.
Mean body mass (g)	933.50	1011.90	980.60	975.3 \pm 39.5
Body mass change (g)	+46.00	-6.80	+3.20	
Dry matter intake				
(g.d ⁻¹)	49.09	52.68	37.97	46.58 \pm 7.67
(g.kg ^{-0.75} .d ⁻¹)	51.69	52.21	38.53	47.48 \pm 7.75
Faecal dry mass				
(g.d ⁻¹)	22.20	25.53	16.86	21.53 \pm 4.37
Apparent digestibility of dry matter (%)	54.78	51.54	55.60	53.97 \pm 2.15
Intake of gross energy				
(kJ.kg ^{-0.75} .d ⁻¹)	1031.35	1086.11	801.51	973.0 \pm 151.01
Faecal energy output				
(kJ.kg ^{-0.75} .d ⁻¹)	574.78	542.43	404.22	507.14 \pm 90.60
Apparent digestibility of gross energy (%)	44.27	50.06	49.57	47.96 \pm 3.21
Intake of digestible energy (kJ.kg ^{-0.75} .d ⁻¹)	456.57	543.68	397.29	465.85 \pm 73.63

$\text{kJ.kg}^{-0.75}.\text{d}^{-1}(\text{n} = 3).$

Intake and Digestibility of Dry Matter Constituents

The intake and digestion of the dry matter constituents of *E. amygdalina* are shown in Table 5.3. The apparent digestibility of total nitrogen may have been reduced by urine contamination of the faeces which could have occurred during the feeding trial. In addition, the true digestibilities of total nitrogen, crude lipid and available carbohydrates are probably lower than the apparent values because of the presence of similar endogenous compounds in the faeces (Cork *et al.*, 1983). Furthermore, the non-structural carbohydrate content of the *E. amygdalina* foliage was estimated by difference (see Methods), therefore it may include other less digestible compounds.

The total phenolics fraction was highly digestible ($92.69 \pm 0.70 \%$). On average, 32% of the neutral detergent fibre (cell wall) was digested. The digestibility of hemicellulose was highly variable because of its determination by difference (see Methods) and the small sample size. Lignin digestibility was particularly high compared with the other cell wall constituents (40%). Chilcott and Hume (1984a) also noted a considerable range of lignin digestibilities (5-47%) in ringtail possums fed on *E. andrewsii*.

5.3.3 Validation of Water Turnover Rates

Water turnover rates estimated using tritiated water were compared with estimated water intake rates over the six day feeding trial (Table 5.4). Preformed water obtained from the *E. amygdalina* foliage was estimated from the fresh weight of leaves consumed by each animal and the water content of the leaves. The volume of water drunk by each animal was measured directly throughout the feeding trial and metabolic water was estimated from the oxidation of the dietary mixture digested for each animal. The composition of this mixture was determined from the total dry matter digested by each animal and the apparent digestibilities obtained for the leaf constituents (Table 5.2 and Table 5.3). The proportion of the digested leaf dry matter made up of total phenolics and absorbed lignin are probably excreted unmetabolised in the urine (Cork *et al.*, 1983; Cork and Sanson, 1990). Therefore the contribution made by total phenolics and lignin to the dry matter digested was ignored in the calculation. Grams of assimilated matter were converted to millilitres of metabolic water assuming 0.39g metabolic water formed per gram of protein, 1.07g metabolic water formed per gram of fat and 0.56g metabolic water formed per gram of carbohydrate (Schmidt-Nielsen, 1975).

Table 5.3 Mean intake and digestion of *E. amygdalina* foliage dry matter by three ringtail possums. Values are means \pm standard deviation.

Constituent	Intake (g.kg ^{-0.75} .d ⁻¹)	Apparent digestibility (%)
Cell contents		
Total nitrogen	0.57 \pm 0.09	54.69 \pm 3.28
Crude lipid	2.53 \pm 0.40	31.37 \pm 2.91
Total phenolics	3.47 \pm 0.57	92.69 \pm 0.70
Available carbohydrates	13.00 \pm 2.10	83.66 \pm 4.04
Cell walls		
NDF	23.73 \pm 3.87	32.91 \pm 4.71
ADF*	22.82 \pm 3.72	32.97 \pm 6.14
Hemicellulose*	0.87 \pm 0.18	35.72 \pm 20.69
Cellulose*	8.82 \pm 1.89	21.89 \pm 3.09
Lignin*	12.96 \pm 2.76	40.41 \pm 8.18

* n = 2

Table 5.4 Comparison of water fluxes determined by materials balance and tritiated water turnover.

Animal	Mean body mass (g)	Preformed water (ml)	Water drunk (ml)	Metabolic water (ml)	Total dietary water ** (ml/kg.d ⁻¹)	Water influx * (ml/kg.d ⁻¹)	Water efflux (ml/kg.d ⁻¹)	Ratio */**
1	1008	402	287.1	55.9	123.18	146.8	147.5	1.19
2	933	363.4	371.5	55.7	141.23	153.9	160.3	1.08
3	974.5	289.6	362.9	47.3	119.74	116.6	116.2	0.97
Mean	971.8	351.7	340.5	52.9	128.05	139.1	141.3	1.08
SD	37.6	57.1	46.4	4.91	11.54	19.81	22.68	0.11

** calculated as preformed water, water drunk and metabolic water.

The daily mean mass specific water intake calculated directly over the six day period was 128.05 ± 11.54 and the daily mean mass specific water intake as estimated by tritiated water turnover was 139.14 ± 19.81 (Table 5.4.). On average, total water intake estimated by tritiated water turnover overestimated the 'actual' water intake by $8.51\% \pm 10.9$ ($n=3$).

5.4 Discussion

The measurements of oxygen consumption made in the thermoneutral zone were considered reasonable estimates of standard metabolic rate in *Pseudocheirus peregrinus viverrinus* since the animals were rested but awake. However, since the possums had not been deprived of food during the night prior to the measurements they were probably not in a post-absorptive state. This is supported by the relatively high RQ values. Therefore, the mean value of standard metabolic rate i.e., 2.8 ± 0.2 watts ($n=20$) should be regarded, more specifically, as resting metabolic rate (as defined by Bligh and Johnson, 1973).

In a comparative study, Kinnear and Shield (1975), investigated the effect of ambient temperature on the metabolic rate and the diurnal variation in metabolic rate and body temperature in the western ringtail possum, *Pseudocheirus peregrinus occidentalis*. They obtained a mean value of $2.54 \text{ W.kg}^{-0.75}$ for the standard metabolic rate of three adult *P. peregrinus occidentalis*. This value is lower than the mean value obtained in this study (i.e., $3.0 \text{ W.kg}^{-0.75}$). However, the possums in Kinnear and Shield's (1975) study were fasted. Therefore the difference between the two values may primarily be related to the extra energy expended in active digestion by the possums in this study. A similar difference in minimum metabolic rate of fasted and non-fasted animals has been noted for another folivorous marsupial, *Petauroides volans* (Foley, 1987). Fasted *P. volans* had a standard metabolic rate of $2.39 \text{ W.kg}^{-0.75}$ (Foley, 1987) whereas the standard metabolic rate of non-fasted *P. volans* was $2.90 \text{ W.kg}^{-0.75}$ (Rubsamen *et al.*, 1984).

Although the standard metabolic rate values obtained for ringtail possums in this study differed from the value obtained by Kinnear and Shield (1975), the minimum body temperature values were similar. However, the thermoneutral zone of the western ringtail possum, *Pseudocheirus peregrinus occidentalis*, was slightly higher than that noted in this study. This difference could be related to the higher

ambient temperatures encountered by a free-living ringtail possum in Western Australia, compared with the cooler climate experienced by possums in Tasmania.

The possums maintained homeothermy, at low ambient temperatures, by reducing their surface area and hence reducing heat loss by curling into a tight ball. Thermoregulatory costs during the cool winter months are probably further reduced in free-living ringtail possums by nest sharing (see Chapter 4). Huddling by small mammals is generally viewed as a means of reducing the thermoregulatory cost of maintaining a high body temperature at a low ambient temperature, by reducing the loss of heat through a reduction of the exposed surface of individuals (Baudinette, 1972). This may in turn confer a further energy saving by extending the range of ambient temperatures at which the group of huddling animals are in thermoneutrality (Fleming, 1980).

At high ambient temperatures evaporative cooling appeared to be facilitated by 'saliva spreading' on exposed extremities. This behaviour has been recorded in several other marsupial species (*Trichosurus vulpecula*, Dawson, 1969; *Megaleia rufa*, Needham *et al.*, 1974; *Petauroides volans*, Rubsamen *et al.*, 1984). Loss of body water through salivation at high ambient temperatures would lead to dehydration if water availability was low. Ringtail possums in the wild suffer weight loss and in extreme cases death over prolonged hot (>40°C) summer days (Pahl, 1987a). This phenomenon is presumably related to the relatively low water content of their leaf diet (see Chapter 4) and a low availability of drinking water in the forest habitat (Degabriele *et al.*, 1978).

McNab (1978, 1986b) proposed that the basal metabolic rate of mammalian species may change in an adaptive manner following the ecological demands with which different species have to cope in their respective habitats. Comparative studies on the basal metabolism of some primate species have provided evidence in support of this hypothesis (Muller, 1985). In particular, folivory appears to be correlated with low basal rates of metabolism in some mammalian species (McNab, 1978). Consequently, McNab (1986a) has suggested that foliage diets may 'lead to' low basal metabolism. However, Foley (1987) points out that this hypothesis is based on values obtained from animals that were not measured under standard conditions or were not strictly folivorous and values that were not the same as those given in the original published study.

Amongst the folivorous marsupial species studied to date only the koala,

Phascolarctos cinereus (Degabriele and Dawson, 1979) and cuscus, *Phalanger maculatus* (Dawson and Degabriele, 1973) exhibit basal metabolic rates which are lower than the rate predicted for marsupials of a similar body mass (Dawson and Hulbert, 1970). The minimum colonic temperature (36.9°C) of the ringtail possum (Kinnear and Sheild, 1975; this study) is similar to the 'mean' marsupial resting body temperature (37.7°C) obtained by Dawson and Hulbert (1970). In addition, the standard metabolic rates recorded by Kinnear and Sheild (1975) and in this study (i.e., 46.6 and 57.8 kcal.d⁻¹, respectively) are slightly higher than the rates predicted for a marsupial of a similar body mass (i.e., 42.9 and 44 kcal.d⁻¹, respectively) from Dawson and Hulbert (1970). Furthermore, two other marsupial folivores, *Petauroides volans* (Foley, 1987) and *Trichosurus vulpecula* (Dawson and Hulbert, 1970) do not show rates of standard metabolism that are lower than other marsupials. It seems increasingly likely that the low basal metabolism shown by some arboreal folivores should be regarded as a preadaptation (Degabriele and Dawson, 1979; Foley, 1987) rather than an adaptation to folivory (McNab, 1978; 1986a).

In common with the leaves from other *Eucalyptus* species, the nitrogen content of the *Eucalyptus amygdalina* foliage offered to the ringtail possums in the feeding trial was low compared with other plant tissue (Cork and Sanson, 1990). The total phenolics content of *E.amygdalina* leaves was at the low end of the range of values reported for other *Eucalyptus* species (summarised in Cork, 1984; Foley and Hume, 1987). However, drying of the leaf samples prior to phenolics extraction, in this study, may have resulted in underestimation of the total phenolics content (Cork *et al.*, 1983). Crude lipid content of *E.amygdalina* leaves was also relatively low compared with values obtained for other *Eucalypt* species (summarised in Cork, 1984) and *Leptospermum laevigatum* which the ringtail possum is known to consume (Chapter 4). The essential oils are included in the lipid content of eucalypt foliage and they vary quantitatively and qualitatively for different species (Foley, 1984 cited in Cork, 1984) Therefore, the relatively low crude lipid content of *E. amygdalina* may reflect the relatively low essential oil content of *E. amygdalina* compared with other eucalypt species (Baker and Smith, 1920).

Total cell wall content was similar to other eucalypt species (Cork, 1984; Foley, 1987; Foley and Hume, 1987). However, the fibre was highly lignified

giving a relatively high lignin : cell wall ratio (Cork, 1984). Digestibility of the cell wall constituent of *E. amygdalina* leaves in this study was lower than that reported by Chilcott and Hume (1984a) for ringtails fed *E. andrewsii* (i.e., 33% and 45%, respectively). Lignin is known to reduce fibre digestibility (Van Soest, 1982), hence this difference may be related to the higher lignin:cell wall ratio of *E. amygdalina* (0.56) compared with that of *E. andrewsii* leaves (0.39).

The energy content of *E. amygdalina* leaves was low compared with other *Eucalypt* species (Cork, 1984). Nevertheless, the digestible energy intake of the ringtail possums in this study ($466 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$) was similar to that of ringtail possums fed *Eucalyptus andrewsii* foliage (i.e., $440 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$, Chilcott and Hume, 1984a). The amount of energy lost in the urine of arboreal marsupials feeding on tree foliage reflects the concentrations and types of essential oils and phenolics present in the foliage (Cork, 1984; Foley, 1987). There is no information on the total phenolic content of *E. andrewsii* foliage fed to ringtail possums in the study by Chilcott and Hume (1984a). However, the essential oil content of *E. andrewsii* and *E. amygdalina* are similar (Baker and Smith 1920). Therefore, urinary energy loss for *Eucalyptus amygdalina* was assumed to be 12% of gross energy intake (Chilcott and Hume 1984a). and the mean intake of metabolisable energy was estimated as $349.09 \pm 57.86 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$ ($n = 3$). This is similar to the estimate of $350 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$ for *Petauroides volans* made by Foley (1987). No allowance was made for the loss of energy in the methane produced during digestion, but, Foley (1987) has shown that this is less than 0.5% of the total gross energy intake in *Petauroides volans* fed *Eucalyptus radiata*.

This value of metabolisable energy may be regarded as the ringtail possums captive maintenance energy requirement (i.e, energy required for minimal activity and synthesis of body chemicals). The maintenance energy requirement of the ringtail possum estimated from this study is 1.3 times the resting metabolic rate (this study) or 1.6 times the basal metabolic rate estimated by Kinnear and Shield (1975) as found for other captive herbivorous species (Hume, 1982). The additional energy requirements of free-living animals appear to be greater multiples of standard metabolic rate than for captive individuals (e.g., Nagy *et al.*, 1979; Smith *et al.*, 1982). The physiological constraints imposed by small body size on energy acquisition from a fibrous diet may be expected to cause problems for the small folivorous ringtail possum if a large increase in food intake was required in order to

meet the the additional energy demands of free existence. These constraints are alleviated in the koala, *Phascolarctos cinereus* by its low standard metabolic rate and correspondingly low metabolisable energy requirements relative to other herbivorous marsupials (Cork *et al.*, 1983). Since the smaller ringtail possum does not appear to have this metabolic advantage it seems reasonable to expect a reduced energy requirement for free-living in the ringtail possum compared with non-folivorous marsupials (see Chapter 6).

Studies of the maintenance energy requirements of animals under controlled conditions, in captivity, are of physiological interest but have little ecological significance. The use of the doubly-labelled water technique to estimate the metabolic rate and water flux of free-living animals (Lifson and McClintock, 1965; Nagy, 1980; Nagy and Costa, 1980) involves a number of basic assumptions (Lifson and McClintock 1965) i.e:

1. Body water volume remains constant during the measurement period,
2. Rates of water flux and CO₂ production are constant through the measurement period,
3. The isotopes label only H₂O and CO₂ in the body,
4. The isotopes leave the body only as H₂O and CO₂,
5. The specific activities of the isotopes in H₂O and CO₂ leaving the body are the same as in body water,
6. Labelled or unlabelled water or CO₂ in the environment does not enter the animal via respiratory or skin surfaces.

Evaluation of these six assumptions by Nagy and Costa (1980) and Nagy (1980) highlighted the associated potential errors which may occur in the use of this technique. However, the magnitude of the errors are small in most situations and can be avoided or minimised by careful choice of experimental procedure (e.g., equilibration and sampling times) and equations (Kunz and Nagy, 1989).

The equations used in this study assumed that pool sizes reflected changes in body mass and that any changes in these parameters were linear during the measurement period (Nagy, 1980; Nagy and Costa, 1980). This method corrects for any changes in mass over time when calculating body water pool sizes for subsequent estimation of water (and CO₂ flux rates, see Chapter 6). Although there were changes in mass during the measurement period for the animals in this study they were not significant, therefore the animals were assumed to be in water and

energy balance.

Several validation trials have been made on a variety of vertebrate species, comparing measurements of metabolic rate and water flux using doubly-labelled water with measurements made using respirometric and energy balance techniques (summarized in Nagy and Costa, 1980 and Nagy, 1989a). These studies show that water flux measurements using isotopic water generally exceed 'direct' measurements made simultaneously, but are generally within $\pm 10\%$ of the 'actual' flux rates (Nagy and Costa, 1980; King and Finch, 1982).

In this study, HTO overestimated 'actual' water intake by $+8.51 \pm 10.9$, $n=3$ (range -3% to 19%). This mean value is within the $\pm 10\%$ range. Nevertheless, the highest value obtained (+19%) is greater than values reported for a variety of mammals by other workers (Nagy and Costa, 1980; King and Finch, 1982). Tritiated water measurements of water flux generally exceed measurements made by balance methods because unlabeled water vapour in the air may move into the animal by diffusion across the lungs and skin (Nagy, 1989a,b). This is measured by tritiated water but not by balance methods. However, the extreme overestimate obtained for one animal in this study is possibly due to loss of the tritium isotope before equilibration (via normal routes of water loss and input of unlabeled water from energy metabolism), resulting in an overestimate of total body water and subsequent water flux rate (King and Finch, 1982; Kunz and Nagy, 1989). In addition, total body water estimates were made from HTO dilution, in this study, and HTO has been found to overestimate total body water by 3 or 4% (Nagy and Costa, 1980). Therefore subsequent estimates of water influx will be overestimated by the same amount.

Errors associated with the measurement of 'food' water and drinking water may also have contributed to the discrepancy between the two methods. Furthermore, errors may have been incurred in the estimation of metabolic water; this suggestion is based on the following considerations. Firstly, the apparent digestibility of total cell contents, total nitrogen and crude lipid may have been lower than their true digestibilities since similar compounds of endogenous origin are normally included in faeces (Cork *et al.*, 1983). This would have lead to an underestimate of metabolic water produced from the oxidation of these constituents. Secondly, the amount of available carbohydrate digested (sugars and starch) was probably overestimated since the available carbohydrate content of the leaves and

faeces were estimated by difference (see Methods). Therefore, this fraction may also have included other leaf constituents such as pectins and water-soluble carbohydrates associated with the leaf cell-walls (Foley and Hume, 1987b). On the other hand, non-structural carbohydrates may form minor components of the total phenolics, crude lipid and crude protein fraction (Cork *et al.*, 1983) which would lead to an underestimate of digested available carbohydrate. Hence, the estimate of digested non-structural carbohydrate and subsequent metabolic water resulting from oxidation of such carbohydrate, in this study, may be close to the true value. However, another error may have arisen from the conversion factor used to convert carbohydrate to metabolic water, since it may vary with type of carbohydrate (Ofstedal, pers. comm.). Thirdly, the probable loss of the phenolics and lignin fraction in the urine was taken into account in the estimation of metabolic water. However, the essential oils component of the digested crude lipid fraction may also have been excreted unmetabolised in the urine (Cork *et al.*, 1983; Cork and Sanson, 1990). Therefore, the proportion of metabolic water estimated from the crude lipid digested may be larger than the actual value.

CHAPTER 6

FIELD METABOLIC RATE, WATER FLUX, AND FOOD CONSUMPTION IN THE RINGTAIL POSSUM.

6.1 Introduction

Studies conducted on eutherian mammals and birds have found annual patterns of the allocation of energy between a variety of behaviours and physiological states e.g., avian systems reviewed in Lack (1968), long-eared owl, *Asio otus* (Winjandts, 1984), brown thornbills, *Acanthiza pusilla* and eastern yellow robins, *Eopsaltria australis* (Haylock and Lill, 1988), golden-mantled ground squirrel *Spermophilus saturatus* (Kenagy *et al.*, 1989) and yellow-bellied marmots *Marmota flaviventris* (Melcher *et al.*, 1989). These patterns appear to have evolved in response to annual changes in environmental conditions (i.e., temperature) and food supply (quality and quantity). These studies have also shown that reproduction is one of the major factors influencing the seasonal pattern of energy allocation in the wild. In mammalian species the female has to cope with the increased energy demands of gestation and, later, producing milk for her young.

Small eutherian mammals in captivity show a substantial increase in metabolisable energy requirement during lactation (e.g., Kaczmarek, 1966; Migula, 1969; Myrcha *et al.*, 1969; Lochmillar *et al.*, 1982 and McClure, 1987). Studies of larger eutherian species have also shown an increased energy requirement for lactation e.g., *Saguinus oedipus oedipus* (Kirkwood and Underwood, 1984), *Erethizon dorsatum* (Farrell and Christian, 1987). However, estimates of metabolism and metabolic costs in controlled laboratory conditions with *ad libitum* food supply can only be extrapolated to the field with caution since laboratory measures exclude the effects of restricted nutrition on lactational performance, the costs of locomotion and the need for the animal to budget time for other activities (Loudon, 1987). Nevertheless, two recent studies of the annual cycle of energy expenditure in natural populations of eutherian mammals have found that the highest total daily energy expenditure of any animal group in the population was that of the females during peak lactation i.e., yellow-bellied marmots, *Marmota flaviventris* (Melcher *et al.*, 1989) and golden-mantled ground squirrel, *Spermophilus*

saturatus (Kenagy, 1987; Kenagy *et al.*, 1989).

Marsupials differ from eutherians in that they bear the bulk of the costs of reproduction during lactation (Tyndale-Biscoe, 1973; Tyndale-Biscoe and Renfree, 1987). Lactation is prolonged in marsupials and can be divided into three Phases (Tyndale-Biscoe and Janssens, 1988, see Chapter 3). The published studies on the energy and/or water requirements of marsupials which include measurements on lactating females indicate that demands are highest during late lactation (Phase 3) when milk production peaks (Hulbert and Gordon, 1972; Kennedy and Heinsohn, 1974; Smith *et al.*, 1982; Green and Eberhard, 1983; Nagy and Suckling, 1985; Lee and Nagy, 1986; Green *et al.*, 1988; Cork and Dove, 1989). Russell (1982) has shown that the pattern of lactation in marsupials (i.e., growth of young, length of lactation) varies between species. Such differences between species may result from different strategies of accomodating maternal energy requirements during lactation.

Digestive adaptations and feeding behaviour are means by which small folivores, such as the ringtail possum, may partly overcome the potential energetic limitations of their small size (see Chapter 1). However, the ringtail possum also appears to show energy-saving strategies, such as reduced activity patterns (see Chapter 4), which may be reflected in its field metabolic rate. Studies, to date, of the field metabolic rate in arboreal folivores (i.e., *Alouatta palliata*, Nagy and Milton, 1979; *Bradypus variegatus*, Nagy and Montgomery, 1980; *Phascolarctos cinereus*, Nagy and Martin, 1985; *Petauroides volans*, Foley *et al.*, 1989) suggest that adaptations to minimise energy expenditure are reflected in low field metabolic rates rather than low basal metabolic rates (McNab, 1978; see Chapter 5).

Published studies on the field metabolism and water requirements of marsupials include some arboreal species; *Gymnobelideus leadbeateri* (Smith *et al.*, 1982), *Phascolarctos cinereus* (Nagy and Martin, 1985), *Petaurus breviceps* (Nagy and Suckling, 1985), *Petauroides volans* (Foley *et al.*, 1989). However, apart from an unpublished study on the ringtail possum in Victoria (cited in Nagy, 1987) there have been no studies detailing the energetics of this species in the wild. Furthermore, few studies have examined the energy and water requirements of free-living marsupials over several seasons.

This chapter examines the seasonal pattern of energy expenditure and water flux in free-living ringtail possums with particular reference to the energy expended

in reproduction by the females. Measurements were made using doubly labelled water (Lifson and McClintock, 1966; see Chapter 5). This method only measures the total energy metabolised (respiratory energy expenditure) by the animal and therefore does not include chemical energy stored as new tissue or exported as milk (Kunz and Nagy, 1988). Estimates were also made of the daily food requirements of ringtail possums, in conjunction with dietary information (Chapter 4 and Chapter 5).

6.2 Methods

6.2.1 Study Area and Animals

The field metabolic rates and water fluxes of wild ringtail possums ($n = 3 - 14$, per trip) were measured on twelve occasions from June 1986 to July 1988 at Whitemark beach, Flinders Island. The study area, timing of field trips and methods used to capture the possums have been described in Chapter 2.

6.2.2 Water Turnover and CO₂ Production

Experimental procedure

Total daily energy expenditure (determined from rates of CO₂ production) and water flux rates were measured using the doubly-labelled water technique (Lifson and McClintock, 1966; Nagy, 1980; Nagy and Costa, 1980) where the differential rates of isotopic hydrogen and oxygen washout are used to calculate water flux and CO₂ production rates (see Chapter 5).

Captured animals were weighed, measured and their sexual status was assessed as described in Chapter 2. An initial blood sample was collected from an ear vein for background isotope levels (see Chapter 5). The animals were then injected intraperitoneally with 1ml tritiated water (HTO: 185 MBq) and 0.3ml H₂¹⁸O (95% + atoms excess ¹⁸O). The injection volumes were calibrated in the laboratory by weighing an equivalent volume of distilled water (assuming 1.0 ml = 1.0 g for distilled water) and was found to be accurate to within ± 0.01 ml. All injections were prepared by the same operator using the same technique. The injected animals were then placed in calico bags and hung on the tree containing the

nest from which they had been caught. Three hours were allowed for the isotopes to equilibrate (see Chapter 5). During this period the animals were undisturbed and they remained quiet.

After the equilibration period a second blood sample was collected, and the animal was fitted with a radio transmitter (Chapter 4). The possum was then released into the tree containing the nest from which it had been captured. Attempts were made to minimise the disturbance time to the animals and to time the procedure so that the animals were released at dusk.

Appropriate recapture intervals are important in isotope studies. Nagy (1983) states that errors in estimating the turnover rates of the isotopes will result if isotope levels are too close to background levels (i.e., sampled too late) or insufficient isotope turnover has occurred (i.e., sampled too early). Sampling intervals can be estimated from multiples of the biological half-life of an isotope. Reliable calculated CO₂ production rates are obtained when the animals are recaptured between one and two half-lives of the ¹⁸O isotope (Nagy 1983). Calculated water fluxes are reliable for longer than calculated CO₂ production rates since HTO is accurate for more than five half-lives (Nagy 1983). The predicted half-life (T_{1/2}) of ¹⁸O as H₂¹⁸O was estimated as 3.24 days (for a 1000g ringtail possum, see Chapter 3) from T_{1/2}=0.151g^{0.444} (Nagy 1983). Therefore, labelled animals were recaptured between four to six days after injection i.e., between one and two predicted half-lives of the ¹⁸O isotope. Actual biological half-life of the ¹⁸O isotope and HTO for the ringtail possums of this study was estimated from the formulae presented by Green and Dunsmore (1978), i.e.,

$$K = \ln C_1 - \ln C_f / t,$$

$$T_{1/2} = 0.693/K.$$

Where K is the fractional turnover rate, C₁ and C_f represent the initial and final isotope activities/concentrations respectively and t is time in days.

On recapture, the animal was weighed, a blood sample was collected and the radiocollar was removed. Attempts were made to recapture the possum at the same time of day as when it had been initially caught to eliminate potential artifacts due to circadian variation in energy metabolism (Kinnear and Shield, 1975). All blood samples were labelled and frozen (-20 °C) until analysed.

Sample analysis

Frozen blood samples were thawed and water was extracted from the whole blood by vacuum sublimation (Vaughan and Boling, 1961). Where only small blood samples had been obtained (i.e., < 0.5ml) water was extracted by heat distillation. An aliquot of each extracted water sample (20 μ l) was then pipetted, using a self levelling capillary tube (Drummond), into a scintillation vial containing 3ml of PCS cocktail (Phase Combining System, Amersham). The extracted water was then assayed for tritiated water activity in a liquid scintillation spectrophotometer (Beckman LS 2800). Aliquots of extracted water (50ml) were placed in Urey tubes together with a standard charge of CO₂ gas. The Urey tubes were incubated overnight at 80°C, after which the equilibrated CO₂ charge was removed and the ¹⁶O:¹⁸O ratio was determined with a VG Isogas 903 isotope ratio mass spectrometer (Gales, 1989).

Prior to each field trip, standard dilutions of the injection solution for both the ¹⁸O isotope and tritiated water were prepared. (i.e., 1 ml of tritiated water was made up to 1000ml with distilled water in a flask and 50ml oxygen-18 was made up to 100ml with distilled water). The standard diluents and animal background blood samples were also analysed for tritium isotope activity and oxygen-18 concentration as described.

Calculations

Total body water pools (TBW) were calculated by comparing blood isotope levels at equilibration to the standard dilutions of the injected isotopes (see Chapter 5). Body water volumes at recapture were assumed to have the same proportion to body mass as at injection. Any change in body mass was estimated as a percentage of the animals mean body mass for the duration of the measurement period.

Rates of CO₂ production, water influx and water efflux were calculated from isotope activities using the equations of Lifson and McClintock (1966, as equation 4), Nagy (1980, as equation 2), Nagy and Costa (1980, as equation 3). Since all the animals in the study were injected with tritium, total body water pool sizes estimated from HTO equilibration samples were used for consistency. It was assumed that changes in body mass reflect changes in pool size and that any changes in these parameters were linear during the experimental period.

6.2.3 Conversion of CO₂ Production to Joules

In order to convert field metabolic rates values from units of carbon dioxide production to standard units of energy metabolism (ie., joules) a thermal equivalent value for CO₂ is required. This value will vary according to the proportions of protein, carbohydrate and fat metabolised from the diet (Blaxter, 1962). Estimation of the proportion of these components metabolised from a leaf diet is complicated by the incomplete knowledge on the composition of each broad fraction (Foley *et al.*, 1989). However, in this study, the conversion factor was based on the apparent digestibilities of *E. amygdalina* leaf constituents estimated from a feeding trial conducted with animals maintained in captivity (see Chapter 5). The contribution made by the leaf constituents to the digestible energy may not necessarily reflect their contribution to metabolisable energy. In particular, the absorbed total phenolics fraction and absorbed lignin are probably not metabolised but are excreted in the urine (Cork *et al.*, 1983; Cork and Sanson, 1990). Therefore, the contribution made by total phenolics and lignin to the dry matter digested was ignored in the following calculations.

From the feeding trial (see Chapter 5) the 0.5397 g of dry matter digested per gram of dry matter ingested contained 0.0411g protein, 0.0167 g crude lipid, 0.2990 g carbohydrate (cell wall and available carbohydrate). Oxidation of this mixture would yield 6.4824 kJ of heat and 0.3053 litres CO₂ (using conversion factors from Gessaman and Nagy, 1988b). This gives a ratio of 21.23 kJl⁻¹ CO₂ which is similar to the ratio determined for the koala, *Phascolarctos cinereus* (Nagy and Martin, 1985) and other plant eating vertebrates (Nagy, 1983). It is also similar to the ratio determined for the greater glider from the respiratory quotients measured in captive animals (Foley *et al.*, 1989).

This conversion factor (21.23 kJl⁻¹ CO₂) was used in the present study to convert field metabolic rate from units of carbon dioxide production to joules. In doing this I have assumed that the digestibility estimates for the various leaf components and dry matter obtained with animals fed *E. amygdalina* are the same for animals feeding on, *Leptospermum laevigatum* in the wild (Chapter 4). The composition of *Leptospermum laevigatum* eaten by ringtail possums in the field (Chapter 4) is slightly different to that of *E. amygdalina* (Chapter 5). Nevertheless, the mean dry matter digestibility obtained for ringtail possums feeding on *E.*

amygdalina (54%, Chapter 5) is within the range of dry matter digestibilities for six ringtail possums fed *L.laevigatum* (53.1% to 60%, Foley and Cork, pers. comm.). Therefore, this assumed conversion factor is considered to be close to the true factor.

6.2.4 Estimation of Feeding Rates

Feeding rates can be estimated in free-living animals from water flux measurements providing the animal does not drink during the measurement period and assuming that there is no pulmo-cutaneous exchange of water. However, ringtail possums were observed drinking water in captivity, therefore, this method was not used. Another method involves estimating the feeding rate required to meet the energy metabolised in the field. Therefore, feeding rates, in this study, are defined as the amount of metabolisable energy required to be consumed for the maintenance of energy balance, and were derived from the following equations (Nagy and Martin, 1985):

$$\text{Metabolisable efficiency} = \frac{\text{energy intake} - (\text{faeces energy} + \text{urine energy})}{\text{energy intake}}$$

$$\text{Rate of energy intake via food} = \frac{\text{FMR}}{\text{Metabolisable efficiency}}$$

Ringtail possums fed *E. amygdalina* in captivity digested $47.96 \pm 3.21\%$ of the gross energy intake (Chapter 5, Table 4.4). The total phenolics content of *L.laevigatum* foliage is lower than *E.amygdalina* foliage (see Chapter 4 and Chapter 5). In addition, essential oil content may be lower in *L.laevigatum* foliage (see Chapter 4 and Chapter 5). Consequently, urinary energy loss was assumed to be only 5% of digestible energy intake in the field animals feeding predominantly on *L.laevigatum*. Underestimation of urinary energy loss will result in feeding rates also being under-estimated.

Therefore, metabolisable efficiency was assumed to be 45% for this study. Feeding rates were calculated using this value and are expressed as units of dry mass and fresh mass of *L.laevigatum* consumed, derived from measurements of

the energy and water content of this foliage (Chapter 4). Feeding rate values have also been expressed per $\text{kg}^{0.67}$ (Nagy, 1987) for comparison with other studies (Table 6.5).

6.2.5 Partitioning of Water Influx

The water influx rates for each group of animals were partitioned into preformed water in the leaves, the water produced during oxidation of nutrients and remaining water (drinking water and water vapour across lung surface).

Preformed water in leaves was derived from the mean water content of *L.laevigatum* foliage (50.89% wet weight, Chapter 4) and the estimated intake of fresh matter (Table 6.5). Calculations of metabolic water production were based on the oxidation of the mixture of protein, carbohydrate and fat digested in the feeding trial (from Chapter 5, presented above) and the rates of CO_2 production measured with doubly labelled water. Using conversion factors from Schmidt-Nielsen (1975) the oxidation of the mixture digested from the leaves would yield 0.0007ml H_2O per ml CO_2 (0.7ml H_2O per litre of CO_2).

To facilitate comparison of data between marsupial species metabolic parameters have been expressed in terms of metabolic body mass as appropriate.(i.e., $\text{kg}^{0.58}$, Nagy, 1987 for field metabolic rate; $\text{kg}^{0.67}$, Nagy, 1987 for feeding rate estimates; $\text{kg}^{0.60}$, Nagy and Peterson, 1988, for water flux)

6.2.6 Statistical Methods

Monthly means were compared using one-way Analysis Of Variance for unequal sample sizes (ANOVA) and the differences were located using the Student Newman-Keuls range test when necessary (Biostat I). Two way Analysis Of Variance (2-way ANOVA) was used to compare differences between sexes over time, or as indicated. Where appropriate, comparisons were made between two means using Student's t-tests (unpaired and two-tailed unless otherwise stated). Percentages were compared after arcsine transformation. Regression equations were calculated by least squares and are presented with the coefficient of determination, r^2 . The 0.05 level of probability was accepted as indicating statistical significance.

6.3. **Results**

6.3.1 **Biological Half-life**

There was no significant difference between the biological half-life of HTO in male ringtail possums i.e., 4.54 ± 6.26 days, $n=51$, and that of HTO in non-lactating females i.e., 4.5 ± 0.68 days, $n=22$ ($df = 71$, $t = -0.204$, $P>0.05$). Similarly, the biological half-life of ^{18}O showed no difference between males i.e., 3.65 ± 0.54 days, $n=43$ and non-lactating females i.e., 3.57 ± 0.68 , $n=22$ ($df = 63$, $t = -0.521$, $P>0.05$). However, the HTO and ^{18}O biological half-lives in lactating females were 4.04 ± 0.57 days, $n=36$ and 3.22 ± 0.3 days, $n=29$, respectively and these differed significantly from those of non-lactating females (i.e., HTO: $df = 56$, $t = 2.845$, $P<0.05$; ^{18}O : $df = 49$, $t = 2.503$, $P<0.05$).

6.3.2 **Body Water, Body Mass and Body Mass Change**

The mean body mass of males varied significantly during the course of the study (Table 6.1; $F_{7,33} = 3.379$, $P < 0.05$). There were two peaks in the body mass of males one in February 1987 and another during January 1988. The lowest body masses for males were measured during May 1987 and June 1988.

The mean body mass of females also varied significantly over time (Table 6.1; $F_{11,53} = 2.420$, $P < 0.05$). The highest values for females were obtained in August 1986 and September 1987 and the lowest value was measured during December 1986. A two-way ANOVA showed no significant difference between the overall mean body mass of male and female ringtail possums ($F_{1,72} = 1.354$; $P > 0.05$) and no significant difference between male and female body mass within a given month ($F_{7,72} = 2.070$; $P > 0.05$). No animals showed significant changes in mass during turnover periods and, therefore, were assumed to be close to energy balance.

Total body water's (TBWs) determined from ^{18}O equilibration were $-1.14\% \pm 3.7\%$ ($n=45$) less than those derived from HTO equilibration samples. However, the differences between the two methods were not found to be significant ($t=1.766$; $P > 0.05$).

TBW and total body mass are expressed as the mean value obtained for the

Table 6.1 Mean body mass and total body water, TBW (derived from tritiated water and expressed as % of body mass) in ringtail possums. Means (\pm SD)

Month	n	Female Mass (g)	TBW (%)	n	Male Mass (g)	TBW(%)
June 1986	3	955 (± 80.5)	79.7 (± 3.4)	—	—	—
August 1986	6	1102 (± 69.2)	64.3 (± 5.9)	—	—	—
October 1986	8	1054 (± 104)	75.7 (± 3.1)	—	—	—
December 1986	2	915 (± 56.6)	73.3 (± 2.5)	1	1020	75.4
February 1987	5	956 (± 139.1)	76.2 (± 3.5)	3	1037 (± 44.8)	75.9 (± 1.4)
March 1987	5	922 (± 98.4)	76.9 (± 6.0)	4	988 (± 8.3)	75.8 (± 6.0)
May 1987	5	938 (± 72.2)	72.6 (± 2.1)	5	954 (± 45.5)	75.1 (± 1.7)
July 1987	6	982 (± 69.9)	74.5 (± 2.4)	5	1012 (± 51.1)	75.1 (± 1.7)
Sept 1987	6	1058 (± 108)	72.0 (± 2.4)	4	955 (± 32.4)	76.4 (± 0.5)
November 1987	8	989 (± 61.5)	82.8 (± 3.3)	6	1009 (± 39.2)	83.4 (± 9.5)
January 1988	6	956 (± 39.4)	78.8 (± 3.1)	8	1053 (± 59.1)	75.3 (± 1.7)
June 1988	6	946 (± 45.7)	71.9 (± 5.0)	6	900 (± 130.6)	71.1 (± 1.7)

males sampled. The TBW of males expressed as a percentage of body mass, varied significantly over the study period (Table 6.1, Figure 6.1a; $F_{7,31} = 2.738$, $P < 0.05$): it was highest during spring (ie., September and November 1987) and lowest during autumn/winter (ie., May 1987, July 1987 and June 1988). The difference between the mean total body mass and mean TBW is made up by body solids (including body fat). Figure 6.1a shows that the proportion of body mass made up by body solids in the males remains similar over the study period except for the spring and early summer months of 1987 when it decreased.

The TBW of the females also varied significantly over the months studied (Table 6.1; $F_{11,51} = 7.290$, $P < 0.05$). The highest value was obtained during November 1987 and the lowest during August 1986. The proportions of body mass made up of body water and body solids in the females are shown in Figure 6.1b. The proportion of body mass made up of body solids in the females was lowest during the spring and summer months. There were no differences between male and female TBW (%) within any month ($F_{7,69} = 1.483$, $P > 0.05$).

During late winter and spring (July, August, September and October) when the mean body mass and the proportion of body mass made up of body solids of the females is at its highest (Table 6.1, Figure 6.1b) most of those females caught were suckling large pouch young (Table 6.2). The data for females were analysed according to the stage of lactation (i.e., Phase 2a, Phase 2b and Phase 3, see Chapter 3) and the size of the young they were suckling. The mean body mass for the females varied significantly according to their stage of lactation ($F_{3,60} = 3.825$, $P < 0.05$). The highest mean body mass was obtained for females at Phase 2b of lactation (1060 ± 96 g, $n=16$) and the lowest was 968 ± 62 g ($n=12$) for females which were suckling small pouch young (Phase 2a, Fig 6.2).

Mean total body water was lowest in females suckling pouch young, (i.e., Phase 2a and Phase 2b of lactation, Fig 6.2). The highest mean value was obtained for females suckling back young (Fig 6.2). In addition the amount of body tissue (g) varied significantly with stage of lactation ($F_{3,62} = 11.434$; $P < 0.05$) being lowest in females suckling back young (Phase 3; Fig 6.2).

Females at Phase 2a of lactation were suckling pouch young which were permanently attached to the teat. It is possible that the injected isotopes equilibrated with the body water of these pouch young which would result in an overestimate of total body water (% body mass) for these females. However, these pouch young

TABLE 6.2 Percentage of lactating and non-lactating ringtail possums measured on each fieldtrip.

MONTH	n	% of total females measured			
		Non-lactating	Phase 2a	Phase 2b	Phase 3
June 1986	3	33			67
Aug 1986	6		33	66	
Oct 1986	8	37		25	50
Dec 1986	2				100
Feb 1987	5	80			20
Mar 1987	5	100			
May 1987	5	80	20		
July 1987	6		66	34	
Sept 1987	6			100	
Nov 1987	8	12		13	75
Jan 1988	6	84			16
June 1988	6		100		

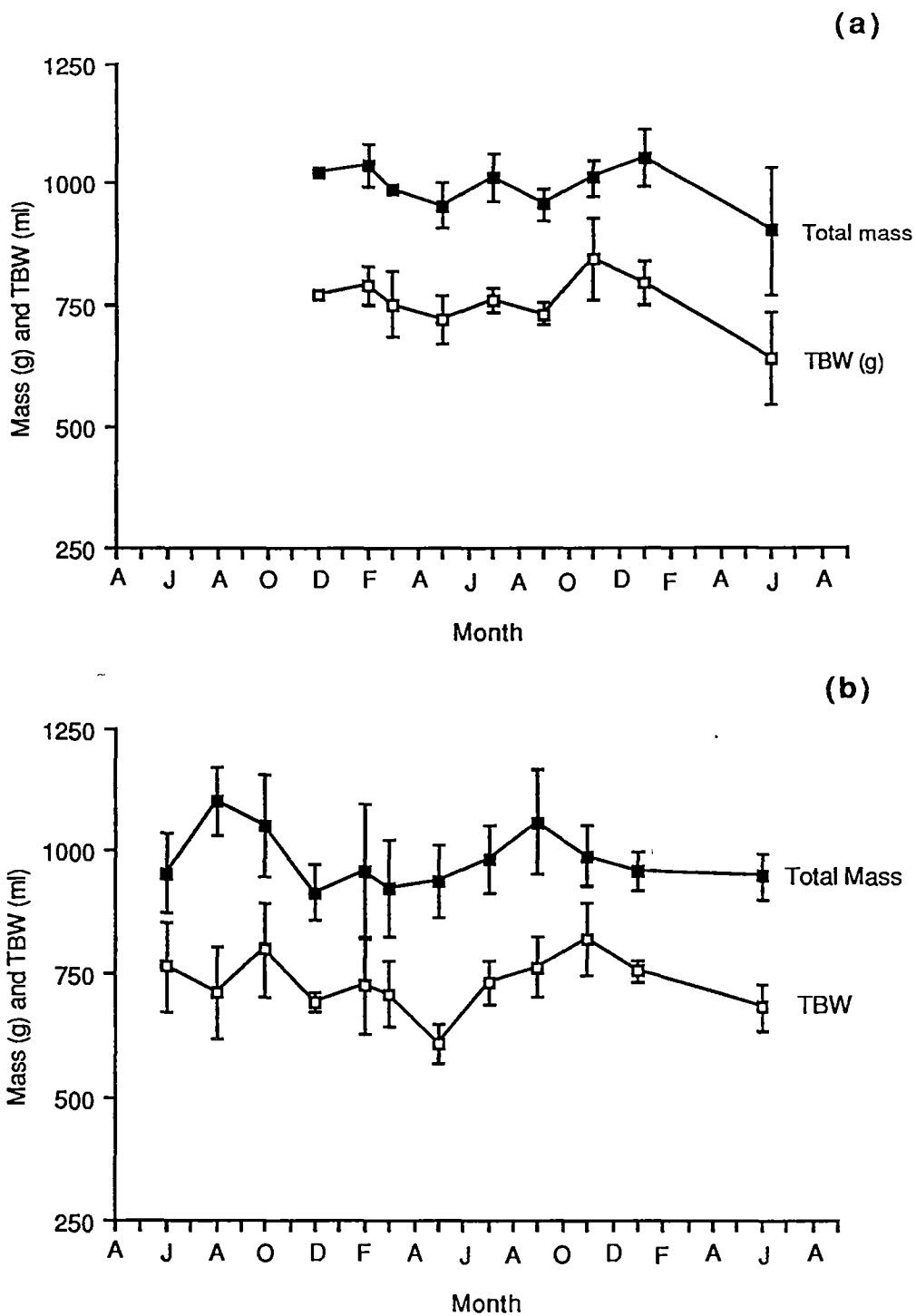


Figure 6.1 Seasonal estimates of the proportion of body mass made up of water and solids in the ringtail possum i.e., (a) Males.(b) Females. Total body water (TBW) and total body mass are expressed as the mean value obtained \pm SD.

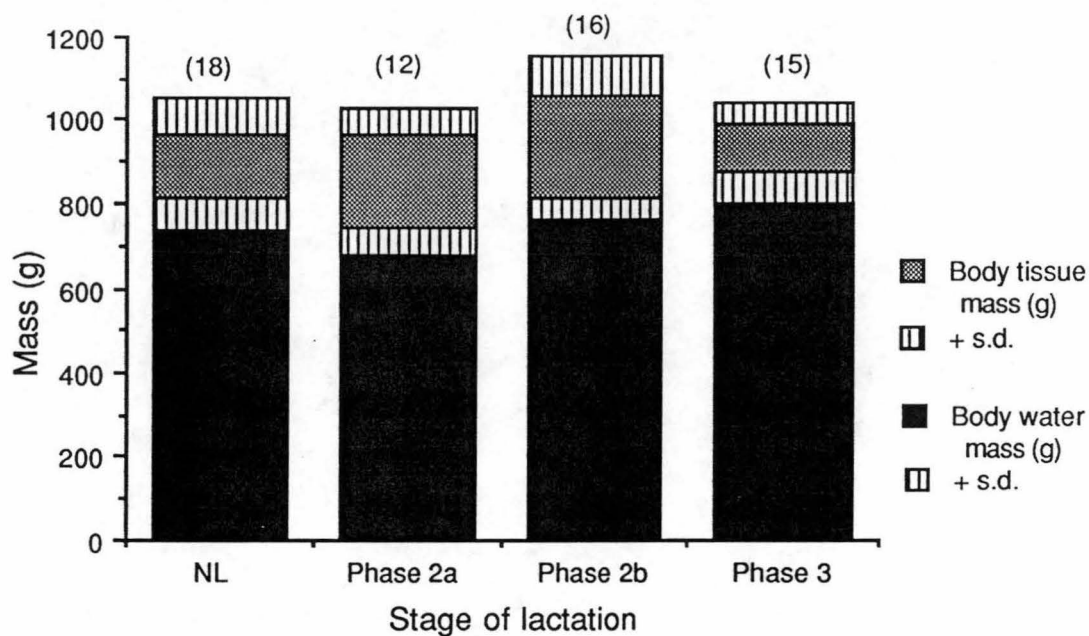


Figure 6.2 Proportion of total body mass made up of body water and body solids in female ringtail possums at different stages of lactation. Values are means +SD for body mass and body water. N is given in parenthesis.

NL = Non-lactating

Phase 2a = Young in pouch and <4.5g.

Phase 2b = Young off teat and in pouch, <51g.

Phase 3 = Young out of pouch between 85g and 453g.

(see Chapter 3)

were <4.5g and therefore, their amount of body water (approx 4ml, see Appendix C) would make little difference to the percentage body water value obtained for the females. The pouch young (weighing between 4.5g and 51g) of females at Phase 2b of lactation had fully released the teat and were probably sucking milk intermittently (see Chapter 7), therefore, it was assumed that the isotopes had not equilibrated with the body water in these young.

6.3.3 Metabolic Rate and Water Flux

Metabolic Rate

The field metabolic rates ($\text{mlCO}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) of adult ringtail possums are shown in Figure 6.3. No reliable estimates were obtained for animals during July 1988 because a malfunction of the isotope ratio mass spectrometer (see methods) resulted in loss of the oxygen-18 samples for this month.

There was no significant variation in the mass-specific field metabolic rates of male ringtail possums throughout the study ($F_{6,26} = 0.704$, $P > 0.05$, Figure 6.3b). Similarly, the metabolic rates of females showed no significant variation over the months studied ($F_{10,46} = 1.583$, $P > 0.05$, Fig 6.3a).

Testis volume (cm^3) was calculated for the individual males sampled in this study and was found to reach a peak which corresponded with the mating season (see Chapter 3). From the values obtained for testis volume and observations on nest sharing (see Chapter 4) those males that were assumed to have been mating during the measurement period were compared with non-mating males. No significant difference was found between the mass specific metabolic rates of these two groups of males ($\text{df} = 30$, $t = 2.002$, $P > 0.05$). The overall mean field metabolic rate for the male ringtail possums from December 1986 to January 1988 was $1.290 \pm 0.322 \text{ mlCO}_2\text{g}^{-1}\text{h}^{-1}$ (Table 6.3)

There were no significant differences between the mass-specific metabolic rates of non-lactating females and females suckling pouch young (i.e., Phase 2a: $\text{df} = 27$, $t=0.752$, $P>0.05$; Phase 2b $\text{df} = 34$, $t=0.544$, $P>0.05$). However, the mean mass-specific metabolic rate of females suckling back young (Phase 3 of lactation) i.e., $1.515 \pm 0.13 \text{ mlCO}_2\text{g}^{-1}\text{h}^{-1}$, $n=14$, was significantly higher than the mean metabolic rate obtained for females not suckling young i.e., $1.213 \pm 0.075 \text{ mlCO}_2\text{g}^{-1}\text{h}^{-1}$, $n = 22$ ($\text{df} = 34$, $t=2.172$, $P < 0.05$; Figure 6.4).

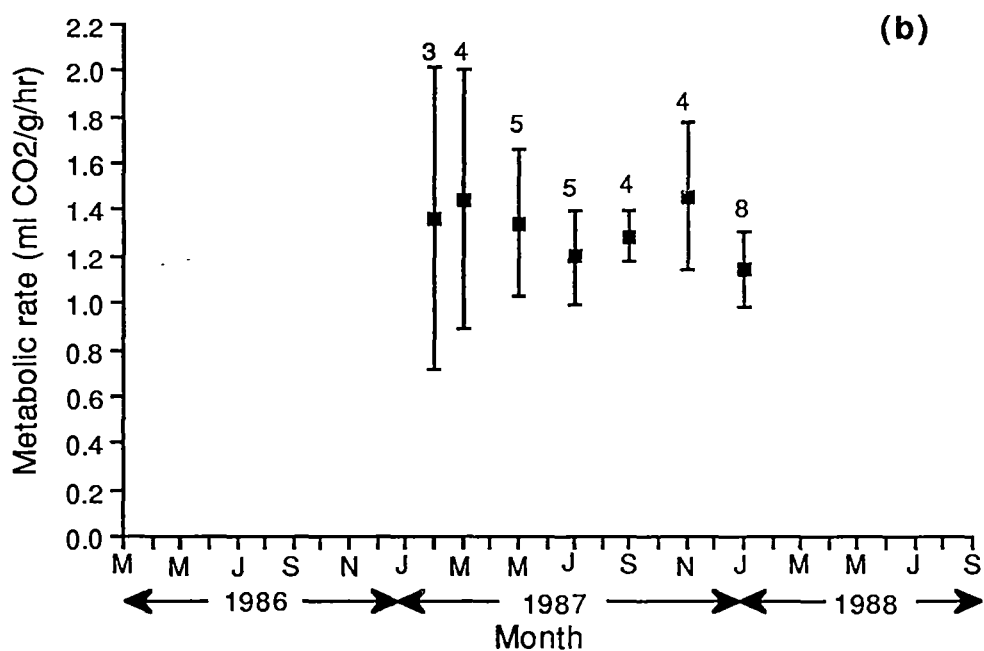
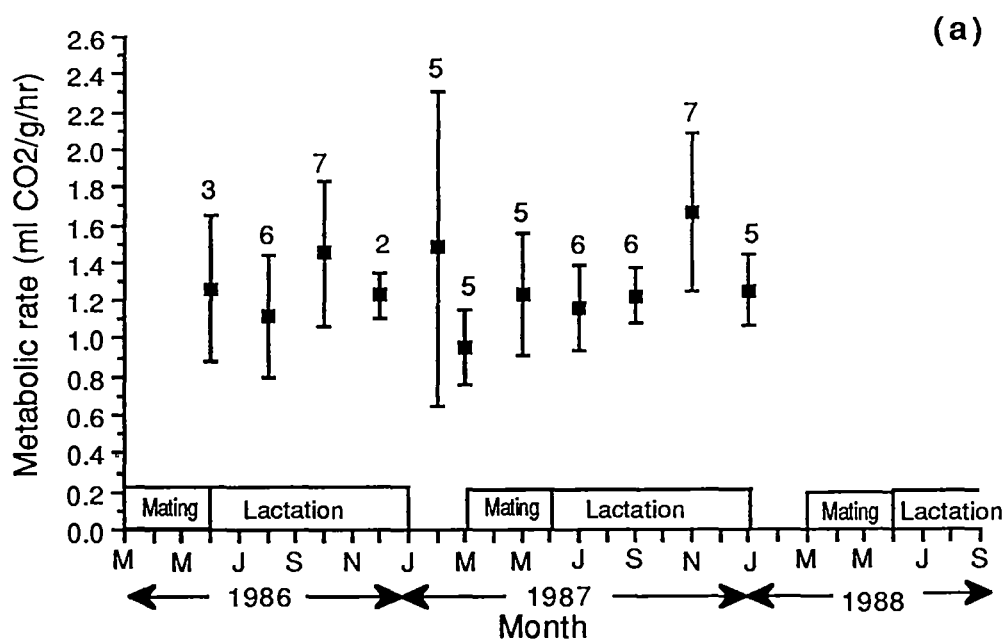


Figure 6.3 Field metabolic rate for free-living ringtail possums; (a) females, (b) males. Values given are means \pm SD, n is indicated above error bars. The timing of mating and lactation are indicated on (a).

Table 6.3 Field metabolic rates, water influx ($\text{ml.kg}^{-1}.\text{d}^{-1}$) and ratio of field metabolic rate to standard metabolic rate in male and non-lactating female ringtail possums. Values are given as means \pm s.d, n is given in parenthesis.

	Mass (g)	H ₂ O Influx ($\text{ml.kg}^{-1}.\text{d}^{-1}$)	($\text{ml CO}_2.\text{g}^{-1}.\text{hr}^{-1}$)	FMR (kJ.d^{-1})	($\text{kJ.kg}^{-0.58}.\text{d}^{-1}$)	Ratio <u>FMR</u> SMR*
Males	990 ± 77.7 (n = 42)	113.8 ± 16.9 (n = 42)	1.290 ± 0.322 (n = 33)	661.88 ± 168.52 (n = 33)	659.11 ± 164.97 (n = 33)	2.5
Females	953 ± 84.8 (n = 23)	115.4 ± 21.1 (n = 22)	1.213 ± 0.35 (n = 22)	584.69 ± 170.65 (n = 22)	643.71 ± 259.25 (n = 22)	2.3

* Standard metabolic rate (see Chapter 5 and text)

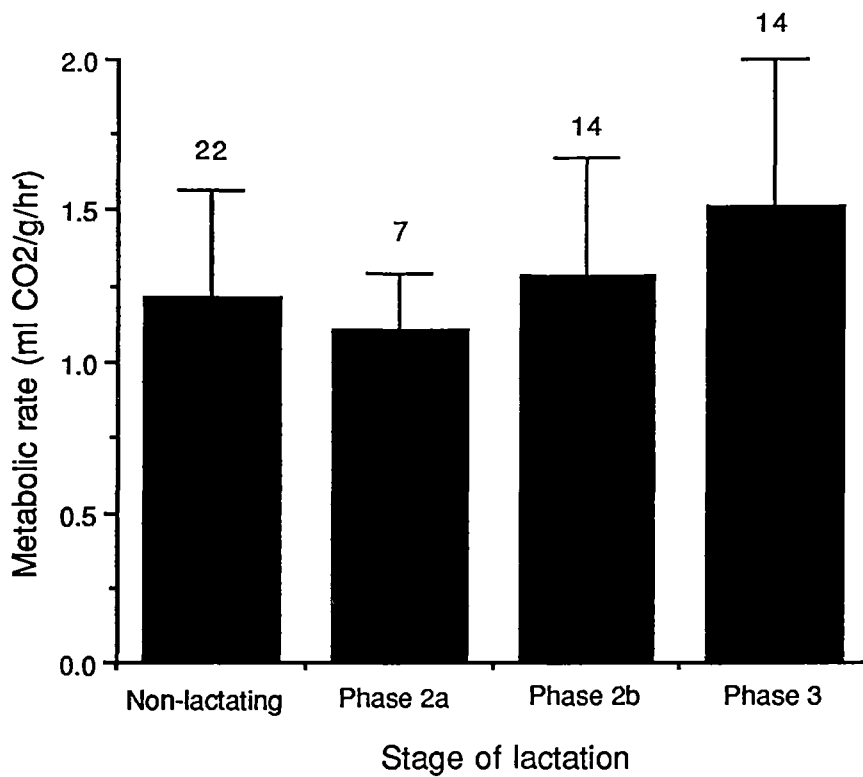


Figure 6.4 Field metabolic rates of female ringtail possums at different stages of lactation. Values are given as means + SD with n above error bars. (see Figure 6.2 and Chapter 3 for definition of Phases of lactation)

No differences were found between male and female mass specific metabolic rates within any month ($F_{6,57}=0.901$; $P>0.05$). Despite the significant seasonal change in body mass in both the male and female ringtail possums, body size did not appear to influence field energy expenditure (Fig 6.5a). Similarly, mass change (%/day) was not correlated with metabolic rate (Fig 6.5b).

The calculated metabolic rates ($\text{kJ}\cdot\text{d}^{-1}$) for ringtail possums based on the conversion factor, $21.23\text{kJ}\cdot\text{l}^{-1}\text{CO}_2$, are presented in Table 6.3 and Table 6.4. The ratio of field metabolic rate to standard metabolic rate (i.e., $265.8\text{kJ}\cdot\text{kg}^{-0.75}\cdot\text{d}^{-1}$, see Chapter 5) has also been presented for each group of animals. This ratio was highest in the females suckling back-young (Phase 3 of lactation) i.e., $2.9 \times \text{SMR}$, and lowest in those females suckling small pouch young (Phase 2a of lactation, Table 6.4). Field metabolic rate was around 2.5 times standard metabolic rate in non-lactating females, females suckling large pouch young (Phase 2b) and males (Table 6.3). The field metabolic rate of lactating females was also compared with the field metabolic rate of non-lactating females (NLFMR). This ratio was highest in females during Phase 3 of lactation; 30% higher than non-lactating females (Table 6.4).

Water Flux

Mean rates of water influx ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) for adult ringtail possums over the months studied are shown in Figure 6.6. Mean water influx rates for male ringtail possums did not vary significantly throughout the study ($F_{7,33}=1.736$, $P>0.05$; Figure 6.6b). Similarly, no significant difference was found between mean water influx rates for those males assumed to be mating during the measurement period and those whose testis volume index suggested they were not mating ($\text{df}=39$, $t=0.947$, $P>0.05$). The overall mean water influx rate for adult male ringtail possums was 113.8 ± 16.9 (SD) $\text{ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ($n = 42$).

Female mean water influx rates, however, varied significantly throughout the study ($F_{11,54} = 10.348$, $P<0.05$). The highest values were obtained during June 1986, October 1986, December 1986 and November 1987 (Figure 6.6a) when a high percentage of the females sampled were suckling back young (Table 6.2).

Figure 6.7 illustrates the mean water influx rates ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) for females at different stages of lactation. There were no significant differences between water influx rates of non-lactating females and those suckling pouch young (i.e., Phase

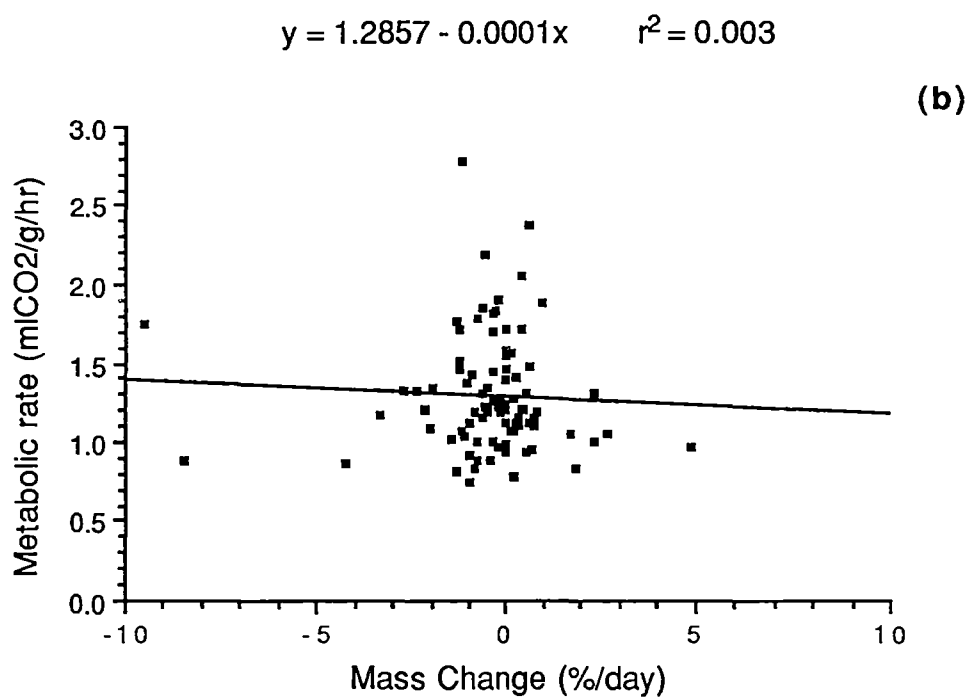
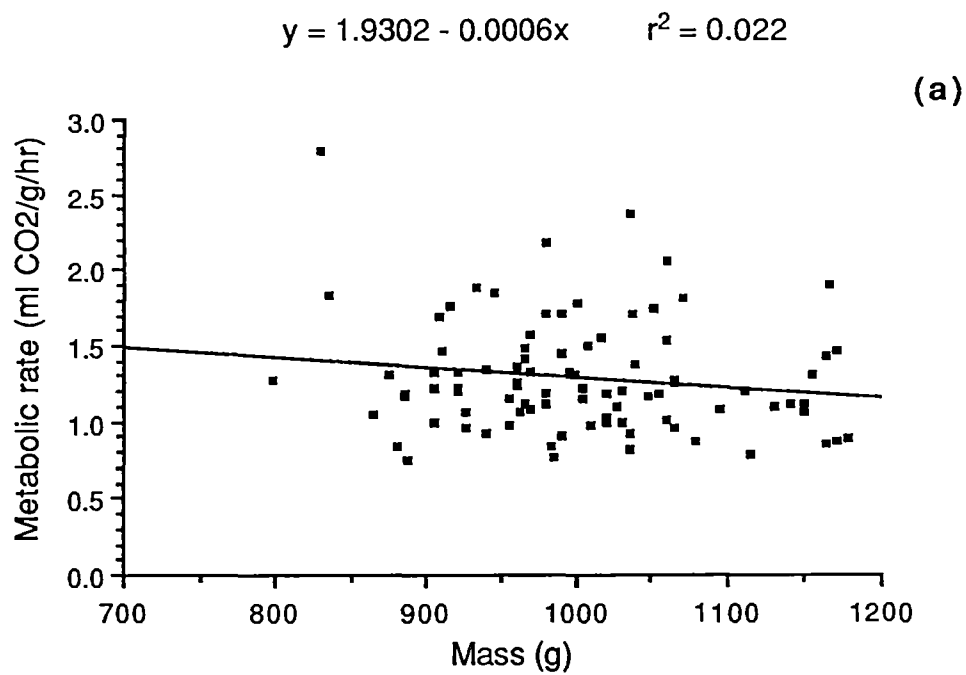


Figure 6.5 Relationship between metabolic rate and (a) body mass, (b) body mass change over the experimental period.

Table 6.4 Body mass and field metabolic rates of lactating female ringtail possums. Values given as mean \pm s.d and n is given in parenthesis.

Lactation Stage	Mass (g)	FMR (kJ.d ⁻¹)	FMR (kJ.kg ^{0.58} .d ⁻¹)	<u>FMR</u> SMR*	<u>FMR</u> NLFMR
2 a	968 \pm 87.26 (n = 12)	550.6 \pm 85.0 (n = 7)	558.34 \pm 33.48 (n = 7)	2.13	0.94
2 b	1059 \pm 96.23 (n = 16)	698.14 \pm 53.26 (n = 14)	670.87 \pm 52.67 (n = 14)	2.50	1.19
3	993 \pm 99.14 (n = 15)	759.19 \pm 58.54 (n = 14)	765.55 \pm 61.48 (n = 14)	2.90	1.29

* standard metabolic rate (see chapter 5 and text)

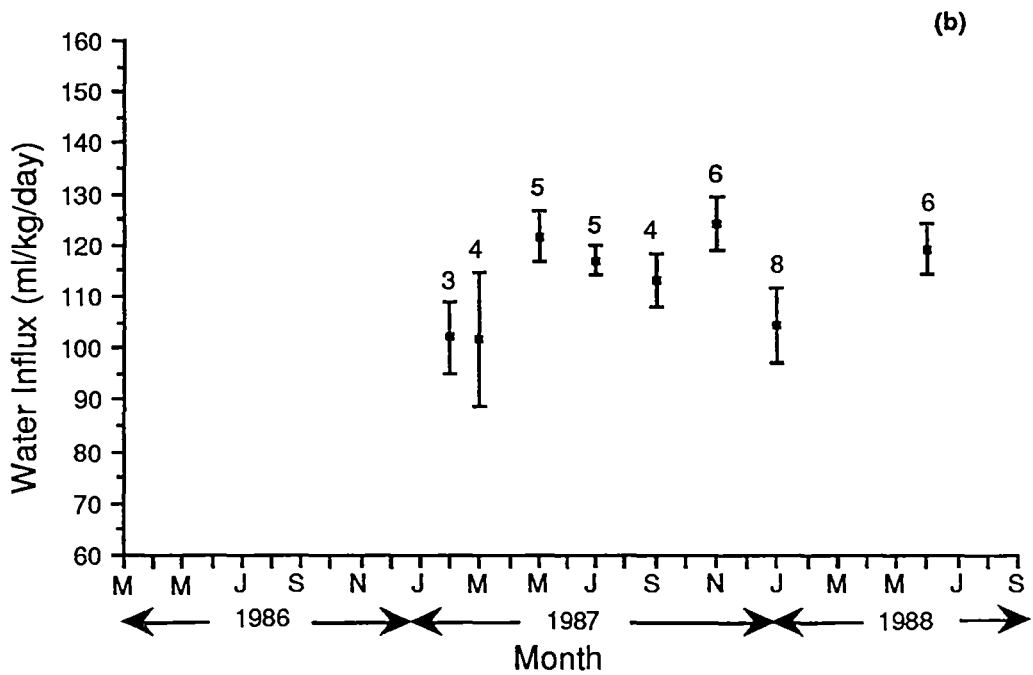
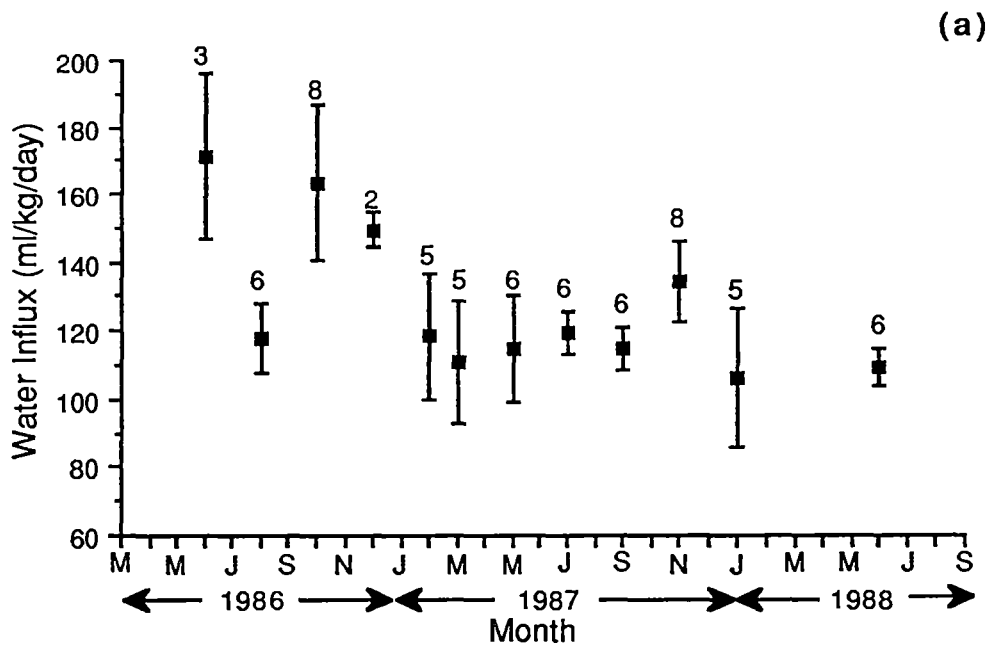


Figure 6.6 Rates of water influx in adult free-living ringtail possums (a) females, (b) males. Values given are means \pm SD, n is indicated above error bars.

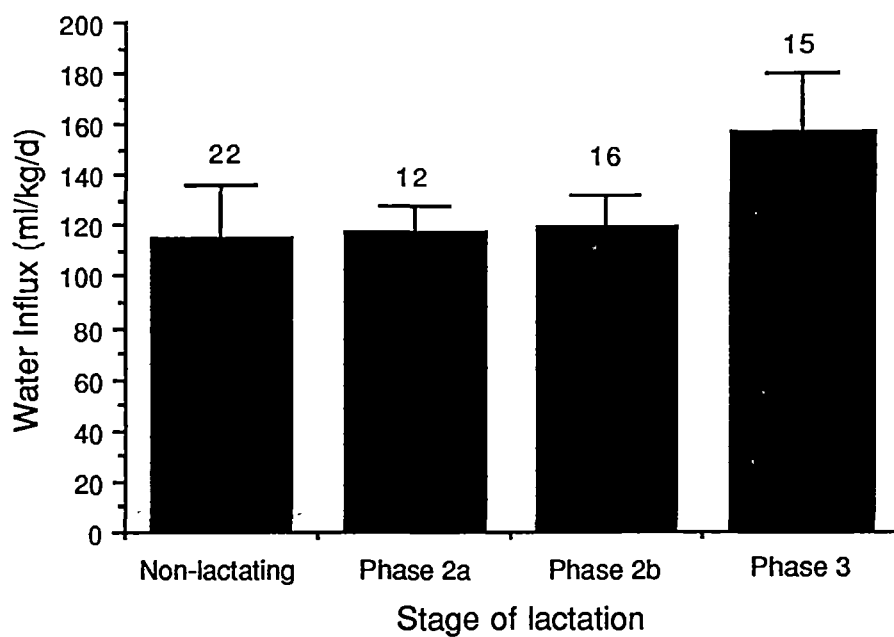
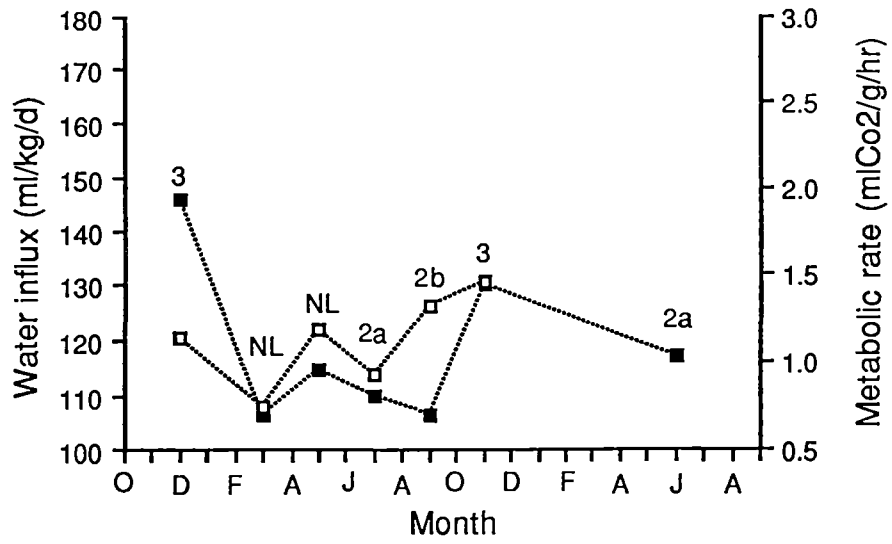


Figure 6.7 Water influx rates of females at different stages of lactation. Means are given \pm SD, n is indicated above error bars.

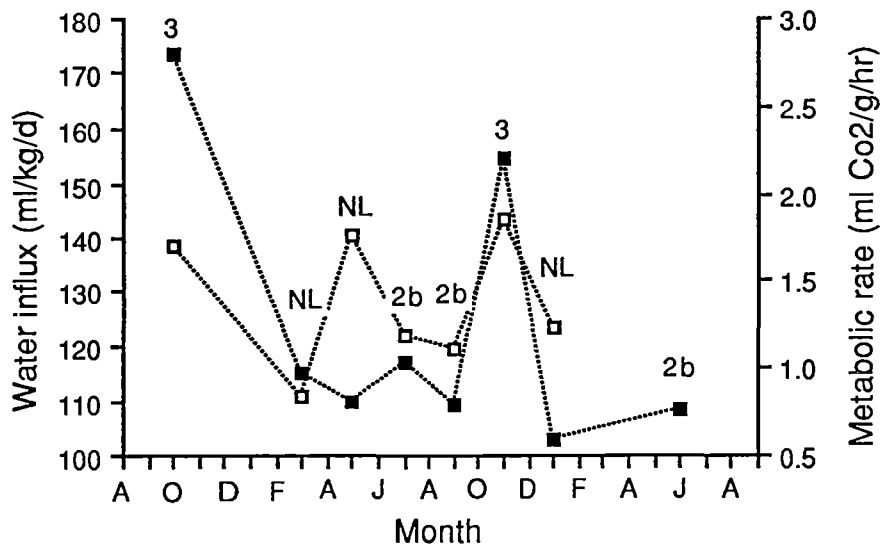
Figure 6.8 Seasonal changes in water influx rates and metabolic rates of three individual ringtail possums, i.e., females F231, F177, F230.

□ = Metabolic rate.
■ = Water influx rate.
NL = non-lactating.
2a = Phase 2a of lactation.
2b = Phase 2b of lactation.
3 = Phase 3 of lactation.

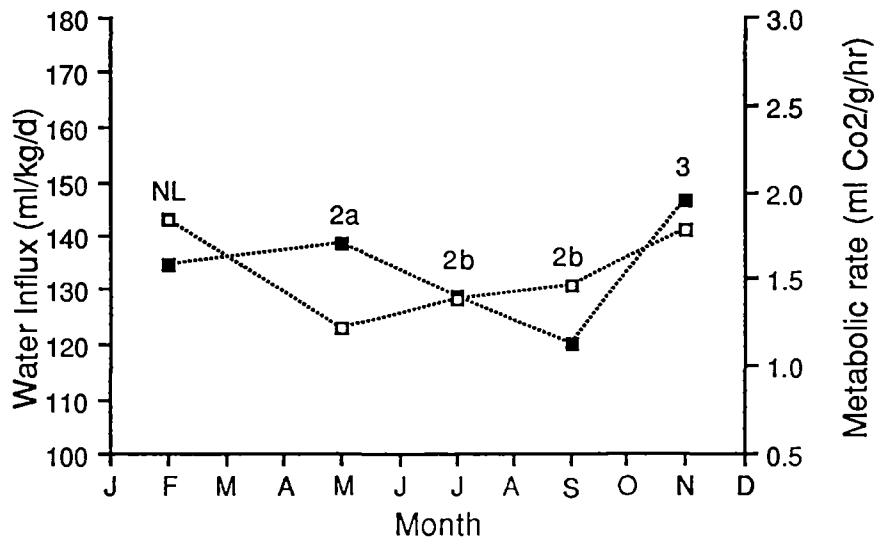
F 231



F177



F 230



2a: $df = 31$, $t=0.320$, $P>0.05$ and Phase 2b: $df = 34$, $t=0.812$, $P>0.05$). However, the mean water influx of females suckling back-young ($156.7 \pm 6.0 \text{ ml.kg}^{-1}.\text{day}^{-1}$) was significantly higher than that of non-lactating females ($df = 35$, $t=5.606$, $P<0.05$). This variation in water influx rate according to the stage of lactation is illustrated for three individual females in Figure 6.8.

Mean rates of water influx did not differ significantly between the sexes during the months in which they were both sampled ($F_{7,72} = 0.943$, $P>0.05$). There was also no significant difference between mean water influx and mean water efflux rates obtained for females within a particular month ($F_{11,102} = 0.08$, $P>0.05$). Similarly, there was no significant difference between water influx and water efflux rates in males within a particular month ($F_{7,66} = 0.045$, $P>0.05$). This reflects the limited changes in body mass during each experimental period and suggests that the ringtail possums maintained water balance during the experimental periods.

Mass specific water influx and CO_2 production rates were not significantly related in male ringtails (least squares linear regression analysis, $t=1.468$, $P>0.05$, Figure 6.9a). However, a significant relationship was found for the females ($t = 2.784$, $P<0.05$, Figure 6.9b); relatively higher metabolic rates are correlated with the ingestion of relatively more water in some individuals as illustrated for three individual females in Figure 6.8 (a,b and c).

6.3.4 The Use of Radio-collars and Metabolic Rate

The water fluxes and metabolic rates of 10 ringtail possums (4 Phase 2a females, 3 Phase 2b females, 1 non-lactating female and 2 males) caught during June, August, October 1986 and June 1988 were measured without using radio-collars. These animals did not change their nest site over the measurement period enabling location and subsequent recapture without radio-collars. The metabolic rates and water influxes obtained for these animals were compared with those of 19 animals that were carrying radio-collars contemporaneously (7 Phase 2a females, 5 Phase 2b females, 2 non-lactating females and 5 males). No significant difference was found between these groups of animals with respect to metabolic rates ($df = 9$, $t = 0.445$, $P>0.05$) or water influx rates ($df = 27$, $t = 1.043$, $P>0.05$). The mean mass change ($\%\text{day}^{-1}$) was -0.402 ± 0.279 (SE), $n = 19$, for those animals carrying collars and -2.148 ± 1.241 (SE), $n = 10$, for those not carrying radio-collars.

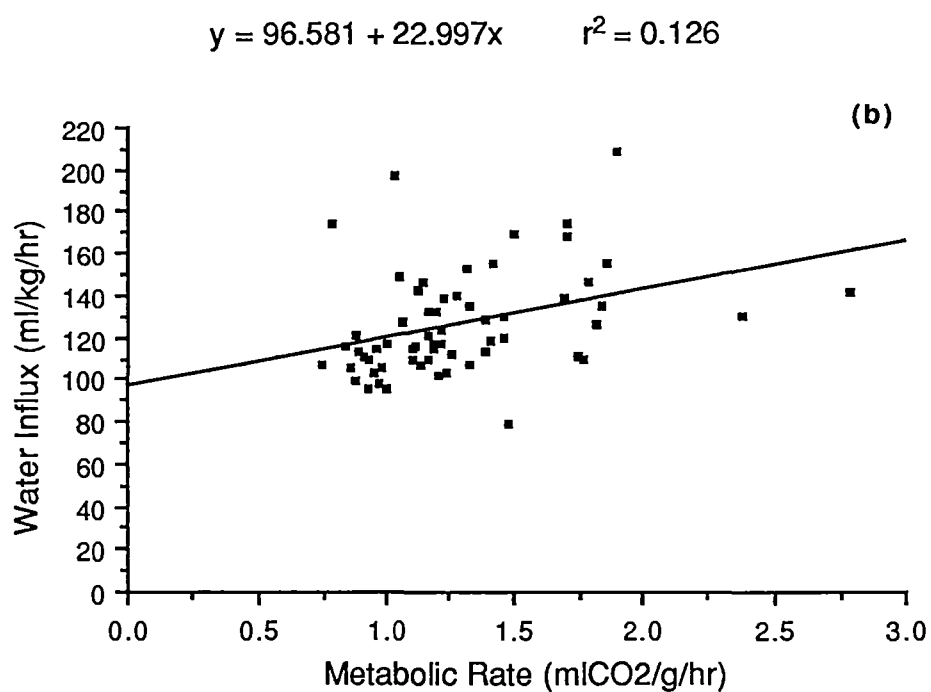
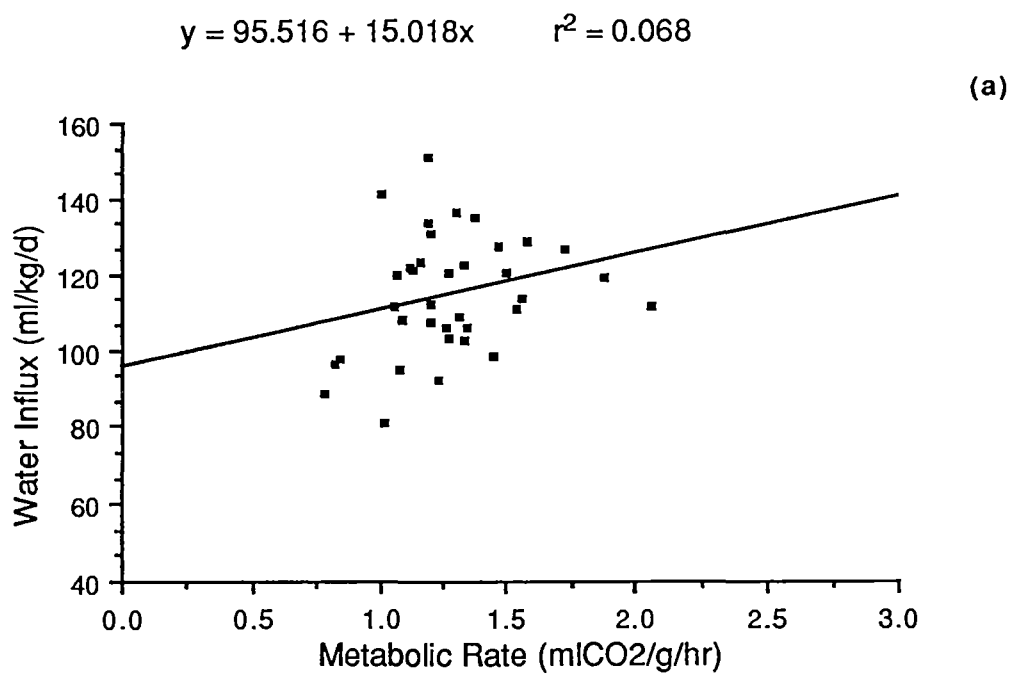


Figure 6.9 Relationship between water influx rates and metabolic rates in (a) male and (b) female adult ringtail possums.

6.3.5 Feeding Rates

The mean gross energy intake for male ringtail possums was 1471 kJ.d^{-1} , which represented a feeding rate of 131g fresh leaves per day (Table 6.5). Females suckling back young had the highest feeding rates (165 g fresh matter per day) and the lowest was for those females with small pouch young (120g fresh matter per day). Females which were not suckling young had a mean gross energy intake of 1426 kJ.d^{-1} and a feeding rate of 127 g fresh matter per day (Table 6.5).

6.3.6 Partitioning of Water Influx

Partitioning of the total water intake is presented in Table 6.6. Rates of water intake are given per $\text{kg}^{0.6}$ for comparison with other species (Nagy and Peterson 1988). The greatest proportion of water intake was made up by free water in the leaves (ie., 55%). Metabolic water represented on average 20%, and the remainder 25% was made up by drinking water (dew or rainwater on leaves, licking wet fur or drinking from a freshwater lagoon within study area). However, a higher proportion of water intake in lactating females was made up by drinking water (Table 6.6).

6.4 Discussion

6.4.1 Potential Errors

The basic assumptions and some of the associated potential errors which may occur in the use of doubly-labelled water to determine metabolic rate and water flux rates have been outlined in Chapter 5. Specifically, potential errors in the estimation of metabolic rate and water flux, in this study, may have arisen from small violations of two underlying assumptions. Firstly, the estimated water flux and CO_2 production rates were assumed to represent the average rates for the measurement period. However, water flux rates and CO_2 production rates may vary over time (Nagy, 1980). Nevertheless, ringtail possums mainly urinate and defecate during the feeding period (Chapter 4) so that water influx and efflux rates vary in parallel. Therefore, body water volumes probably remained relatively constant over time,

Table 6.5 Estimated feeding rates of free-living ringtail possums (Means \pm SD).

	(kJ.d ⁻¹)	(kJ.kg ^{-0.58} .d ⁻¹)	(gdry matter.d ⁻¹)	(g.kg ^{-0.67} .d ⁻¹)	(gfresh mass .d ⁻¹)
Males	1470.8 \pm 374.5 (n = 33)	1478.6 \pm 389.1 (n = 33)	64.7 \pm 16.5 (n = 33)	65.0 \pm 17.2 (n = 33)	131.0 \pm 33.4 (n = 33)
Non-lactating females	1299.3 \pm 379.2 (n = 22)	1262.5 \pm 386.6 (n = 22)	57.2 \pm 16.7 (n = 22)	55.4 \pm 17.1 (n = 22)	115.7 \pm 33.7 (n = 22)
Lactating females					
Phase 2a	1223.5 \pm 188.9 (n = 7)	1208.3 \pm 193.4 (n = 7)	53.8 \pm 8.3 (n = 7)	53.1 \pm 8.5 (n = 7)	109.0 \pm 16.8 (n = 7)
Phase 2b	1551.4 \pm 442.8 (n = 14)	1617.6 \pm 457.1 (n = 14)	68.3 \pm 19.4 (n = 14)	71.7 \pm 20.3 (n = 14)	138.2 \pm 39.5 (n = 14)
Phase 3	1687.1 \pm 486.7 (n = 14)	1679.4 \pm 497.5 (n = 14)	74.3 \pm 21.4 (n = 14)	73.9 \pm 22.0 (n = 14)	150.3 \pm 43.4 (n = 14)

Table 6.6 Partitioning of water intake in free-living ringtail possums (Means \pm SD).

	Total Influx		Preformed in food		Metabolic H ₂ O		Difference	
	(ml.d ⁻¹)	(ml.kg ^{-0.6} .d ⁻¹)	(ml.d ⁻¹)	(ml.kg ^{-0.6} .d ⁻¹)	(ml.d ⁻¹)	(ml.kg ^{-0.6} .d ⁻¹)	(ml.d ⁻¹)	(ml.kg ^{-0.6} .d ⁻¹)
Males	112.4 ± 16.8 (n = 42)	113.1 ± 15.9 (n = 42)	66.7 ± 16.9 (n = 42)	66.4 ± 16.7 (n = 33)	21.82 ± 5.6 (n = 33)	21.7 ± 5.4 (n = 33)	24.3 ± 27.8 (n = 33)	24.2 ± 27.7 (n = 33)
Non-lactating females	109.1 ± 19.6 (n = 22)	112.7 ± 19.8 (n = 22)	58.9 ± 17.1 (n = 22)	60.8 ± 17.4 (n = 22)	19.3 ± 5.6 (n = 22)	19.8 ± 5.7 (n = 22)	30.9 ± 21.7 (n = 22)	31.9 ± 22.6 (n = 22)
Lactating females								
Phase 2a	113.5 ± 13.1 (n = 12)	115.6 ± 11.0 (n = 12)	55.4 ± 8.5 (n = 7)	56.3 ± 8.9 (n = 7)	18.15 ± 2.8 (n = 7)	18.4 ± 2.9 (n = 7)	46.4 ± 14.3 (n = 7)	46.8 ± 13.1 (n = 7)
Phase 2b	126.9 ± 16.7 (n = 16)	122.5 ± 13.0 (n = 16)	70.3 ± 20.1 (n = 14)	67.6 ± 19.9 (n = 14)	23.0 ± 6.6 (n = 14)	22.1 ± 6.5 (n = 14)	34.7 ± 29.0 (n = 14)	32.8 ± 27.1 (n = 14)
Phase 3	157.7 ± 35.5 (n = 15)	156.6 ± 27.5 (n = 15)	76.5 ± 22.1 (n = 14)	77.2 ± 23.2 (n = 14)	25.0 ± 7.2 (n = 14)	25.3 ± 7.6 (n = 14)	56.8 ± 44.1 (n = 14)	55.5 ± 42.9 (n = 14)

resulting in only small errors caused by unequal rates of influx and efflux (Nagy and Costa, 1980, Nagy, 1980). Secondly, it was assumed that no labelled water or CO₂ in the environment entered the animal over the measurement period. Nevertheless, recycling of labelled CO₂ and/or labelled water from pockets of air confined in the nest may have led to errors in the estimation of CO₂ production and/or water flux rates in some animals (Nagy, 1980; Nagy and Costa, 1980). However, the ringtail possums' nest is constructed of a loose arrangement of twigs and dead foliage (personal observation) which probably minimises air flow but would not restrict it completely. In addition, blood samples taken from unlabelled animals sharing a nest with a labelled animal revealed no significant transfer of isotopes.

Although the doubly labelled water technique used in this study was not validated (see Chapter 5), a study on little penguins, *Eudyptula minor*, found only a small difference (+1.75%) between estimates of metabolic rate from the energy balance method and those obtained using doubly labelled water and the same experimental procedures used in the present study (Gales, 1989). Furthermore, Nagy *et al.* (1989) recently validated the use of doubly labeled water in a folivorous marsupial, *Petauroides volans*. Using a respirometric technique, they found the DLW technique underestimated CO₂ production in this marsupial by 8.3% on average, which is within the range of mean errors found in other validation studies (Nagy, 1980; Nagy 1987). Therefore, it seems reasonable to assume that the values for CO₂ production in the ringtail possum determined here using the doubly labelled water technique are sufficiently accurate for comparison with other field studies.

The sources of discrepancies found in laboratory validation trials may compound in field measurements because of additional potential errors in this situation (Nagy, 1980). For example, one potential source of error in the field involves the use of radio-transmitters. The assumed standard that limits the weight of a radio transmitter is 5% of the animals body mass (Macdonald, 1978). In using this 5% upper limit biologists assume that the transmitter will not significantly alter the animals normal behaviour and daily energy metabolism. However, a study on homing pigeons, *Columba livia*, using doubly-labelled water found that CO₂ production was significantly greater in birds wearing transmitters (Gessaman and Nagy, 1988a). Nevertheless, the use of radio-collars did not appear to alter the daily

energy metabolism or water flux of the ringtail possums in this study.

The procedures used in this study were standardised as far as possible, hence although there may be slight errors in the actual values estimated, any biases will be consistent between individual animals, animal groups and seasons.

6.4.2 Total Body Water and Condition

The physical wellbeing of an animal is generally described as 'condition' and can be estimated by a variety of morphological (Bamford, 1970; Hocking, 1981; Bakker and Main, 1980) and physiological methods (Barnett *et al.*, 1979). Indices of condition using percentage total body water estimation are based on the observation that total body water is inversely proportional to the percentage of fat in a mammal's body (Pace and Rathburn, 1945; Searle, 1970; Green and Eberhard, 1983; Hulbert and Grant, 1983). Despite the range of techniques, no one method seems acceptable as a standard. Such a standard would enable comparisons between populations in different habitats (reviewed by Humphreys *et al.*, 1984). In this study total body water estimates and subsequent estimates of total body solids, which includes body fat, relative to body mass were considered adequate to assess the body condition of ringtail possums between seasons and groups.

Estimates of total body water (TBW) derived from tritium dilution are generally higher than those measured directly by carcass desiccation (Rothwell and Stock, 1979; Bakker and Main, 1980; Nagy and Costa, 1980; Green and Eberhard, 1983). No animal was killed in this study to obtain measured values, therefore the actual values of TBW may be slight overestimates. Estimates of TBW based on ^{18}O dilution more closely represent desiccated water space than those estimated by HTO dilution (Nagy 1980). In this study TBW determined from HTO dilution was higher than TBW determined from ^{18}O dilution, but the difference was not significant. However, any biases which may have occurred will be consistent between groups and will, therefore, not affect comparisons of TBW between months or groups.

The TBW (% body mass) estimates of ringtail possums suggest that, in general, they are lean animals. Similarly, Pahl and Lee (1988), when comparing groups of ringtail possums, noted that monthly variations in an index of 'condition' (i.e., body weight/head length) and monthly variations in body weight showed little difference. They related this observation to the probable lack of fat reserves in

ringtail possums. Another arboreal folivore, *Phascolarctos cinereus*, also has a lean carcass with its large caecum holding significant amounts of moist material (Degabriele *et al.*, 1978). Such relatively low levels of fat reserves, indicated by high levels of body water, are a characteristic of many arboreal mammals (Table 6.8). It has been suggested that this is an adaptation to life in the trees, as the extra weight of large fat deposits would make movement in the tree canopy difficult for an arboreal vertebrate (Janzen, 1978).

Environmental variables, such as food availability, and endogenous reproductive factors may all affect the condition of a particular animal. Seasonal fluctuations in condition have been reported for a number of herbivorous mammal species in the temperate zone e.g. rabbits, *Oryctolagus cuniculus* (Martin, 1977); red deer, *Cervus elaphus* (Caughley, 1971); *T.vulpecula* (Barnford, 1970; Hocking, 1981). Although based on a variety of condition indices these fluctuations typically show a winter/spring decline with a peak during the summer and autumn. This 'standard' pattern is not very well defined in the ringtail possum.

Female ringtail possums showed a more pronounced fluctuation in 'condition' than the males. As indicated by their total body solids content, the females appeared to be in good condition immediately prior to emergence of the young from the pouch (late winter/early spring) when their body mass was greatest. It seems likely that the female ringtail possum accumulates small fat reserves during early lactation which can then be drawn upon during late lactation. Similarly, Bell (1981) proposes that female *T.vulpecula* in New Zealand lay down fat reserves in autumn and early winter (where possible) and utilize them in late winter and early spring when the young are growing rapidly and demands of lactation are highest. This scenario has also been suggested for the Tasmanian Devil *Sarcophilus harrissi* (Nicol, 1978). However, the increase in total body solids seen in the female ringtail possums during early lactation may only partially be a result of fat reserves due to the increase in size of the mammary gland at this stage (see Chapter 7).

The mass-specific water contents of females peaked during the late spring months. This was similar to that observed in the males. However, the females remained in poor condition throughout spring into early summer over the period when the majority were suckling back-young. This suggests that the energy demands of late lactation (Phase 3) were affecting the condition of female ringtails at this time. Increases in mass specific total body water space and decrease in the fat

reserves (or condition) have been noted in other marsupial species during the later stages of lactation; e.g., *Trichosurus vulpecula* (Bamford, 1970; Kennedy and Heinsohn, 1974); bandicoot, *Isodon macrourus* (Hulbert and Gordon, 1972); rock-wallaby, *Petrogale inornata* (Kennedy and Heinsohn, 1974); eastern quoll, *Dasyurus viverrinus* (Green and Eberhard, 1983).

The differences between female and male ringtail possums in the seasonal pattern of 'condition' and body mass suggest that reproductive factors such as lactation are influencing 'condition' to a greater degree than environmental factors. This conclusion is supported by the similar seasonal cycle in body mass of ringtails in captivity which were fed *ad libitum* (Appendix D). Supplementary feeding of other mammals during the weight loss period of their cycle has also failed to alter their natural cycle, e.g., *Peromyscus maniculatus* (Stebbins, 1978), *Rattus fuscipes* (Stewart and Barnett, 1983).

6.4.3 Energy metabolism

Differences in the rate of energy metabolism may reflect the kinds of food resources used by different species of mammals (McNab, 1980). Nagy (1987) allometrically compared the field metabolic rates, measured using doubly labelled water, of herbivorous and non-herbivorous mammals. He found that, in general, herbivores have lower field metabolic rates than nonherbivores. Authors have suggested that arboreal folivores, in particular, live energetically conservative lifestyles (Eisenberg, 1978; McNab, 1978). More specifically, Hume *et al.* (1984) suggest that a low ratio of field metabolic rate to basal metabolic rate is a characteristic of arboreal folivorous marsupials. This hypothesis is supported by the limited direct data on energy expenditure in free-living arboreal marsupials. A particularly low ratio is found in eutherian folivores (i.e., three-toed sloth, *Bradypus tridactylus* and howler monkey, *Alouatta palliata* see Table 6.8). In this study, the overall mean values obtained for the metabolic rate of a 1 kg male ringtail possum and a 1kg non-lactating female i.e., 659 kJ.d⁻¹, 644 kJ.d⁻¹ respectively, are similar to that predicted allometrically for a 1kg marsupial; i.e., 630 kJ.d⁻¹ from Nagy (1987). However, this predicted value is derived from measurements made on a relatively small range of marsupial species. When expressed as a multiple of basal metabolism the ringtail possums' field energy expenditure was lower than the values

Table 6.8 Energy and water metabolism in free-living arboreal mammals measured using doubly labelled water (mean values for adult males only unless stated otherwise); i.e., (*) average value for males and females, (#) average value for all animals over the months studied.

Species	Diet **	Mass (g)	Field metabolic rate (kJ.d ⁻¹)	<u>FMR</u> SMR	Total Body Water (% body mass)	Water influx rate. (ml.kg ^{-0.6} .d ⁻¹)	References
<i>Pseudocheirus peregrinus</i> (#)	F	990	655	2.5 ^{""} 3.0 ["]	76%	114	This study
<i>Phascolarctos cinereus</i>	F	10800	2031	2.6	73.6%	114	Nagy and Martin(1985)
<i>Petauroides volans</i>	F	1050	557	2.7	71.6%	88	Foley <i>et al</i> (1989)
<i>Trichosurus vulpecula</i> (*)	F	1520			68.6%	70	Kennedy and Heinsohn (1973)
<i>Bradypus variegatus</i>	F	4450	775	1.7	70.0%	74	Nagy and Montgomery (1980)
<i>Alouatta palliata</i> (*)	F/Fr	6460	3146	2.0	-	-	Nagy and Milton (1979)
<i>Gymnobelideus leadbeateri</i> (#)	I/E	129	225	5.8	-	126	Smith <i>et al</i> (1982)
<i>Petaurus breviceps</i>	I/E	135	192	3.9	70.1	100	Nagy and Suckling (1985)

**F = folivore, F/Fr = Folivore/frugivore, I/E = Insectivore but also consumes plant exudates.

" = Using BMR from Kinnear and Shield (1975). "" = Using SMR from Chapter 5.

available for marsupial omnivores and similar to values found for other folivorous marsupials (see Table 6.8). This relatively low energy expenditure for free-existence is consistent with the ringtail possums' conservative activity budget (Chapter 4).

In general, fluctuations in energy allocation related to environmental factors (weather, food quality) may be expected to be less pronounced in animals inhabiting areas where seasonal variations in the climate are not particularly great. Studies have shown that passerine birds in southern temperate forests show only a moderate increase in daily energy expenditure during winter months compared with birds inhabiting forests in the north temperate zone where the winters are more severe (Magrath and Lill, 1983; Haylock and Lill, 1989). In the present study, the comparatively mild winters experienced on Flinders Island (see Chapter 2) may be the reason for the lack of a significant increase in energy expenditure by the ringtail possums during the winter. In addition, increased insulation (pelage density) and behavioural adaptations, such as nest usage and sharing (see Chapter 4) may reduce the ringtail possums' energy expenditure on heat production.

Energy is needed for reproduction in excess of energy required for maintenance, thermoregulation and other activities. Mating behaviour in the ringtail possum can involve prolonged copulation and is often accompanied by frequent vocalisation (Perry, personal communication). Males may also have to fend off other males interested in the receptive female and during the breeding season may move about more actively in search of oestrous females (Perry, personal communication). In view of this behaviour an increase in the males daily energy expenditure during the mating season was anticipated. However, there was no evidence, to suggest that mating in male ringtails resulted in a substantial increase in energy expenditure. Nevertheless, it should be pointed out that only an indirect estimation of a males potential to mate (i.e., testis size) was used in this study. Therefore, it was difficult to determine if a particular male had in fact copulated during a specific measurement period. However, studies of field metabolic rate in male *Antechinus stuartii* and male *Antechinus swainsonii* (Lee and Nagy, 1986; Crowley, 1989) also found that mating was not associated with significant increase in energy expenditure.

In contrast to the males, reproduction resulted in a significant increase in energy expenditure by the females. The daily energy expenditure by the mother varied according to the stage of lactation. Females suckling pouch young which had

not released the teat. (Phase 2a of lactation.) had the lowest daily energy expenditures (-13% of non-lactating females field metabolic rate). This early stage of lactation in the ringtail possum, as in other marsupials, is characterised by the production of small quantities of milk to feed very small young (Green, 1984; Green *et al.*, 1988; see Chapter 8). However, after the young had left the pouch (Phase 3) the females energy expenditure increased significantly. Since milk production reaches a peak at this time (Green *et al.*, 1988; see Chapter 8), this probably results from the increased energy required for milk synthesis and production. In addition, aspects of parental care may place less obvious energy demands on females at this time, such as the burden of carrying the young on her back.

The limited information on total daily energy expenditure in lactating marsupials suggests a similar pattern of energy expenditure according to stage of lactation (see Table 6.7). Energy expenditure during late lactation has only been measured in two other natural populations of marsupial, *A. stuartii* and *A. swainsonii* (Lee and Nagy, 1986; Crowley, 1989). The highest increase in daily energy expenditure was found for *A. swainsonii* females during late lactation (+72%). Green and Eberhard (1983) estimated the energy requirements of *Dasyurus viverrinus* from food consumption estimates and found those of late lactation females to be +83% greater than that of non-lactating females. These increased energy requirements are significantly higher than that estimated in this study for lactating ringtail possums (+18%, see Table 6.7). However, a similar relatively conservative increase in energy requirements during lactation has been noted for an arboreal primate, *S. oedipus oedipus*, when compared with other eutherian mammal species (Kirkwood and Underwood, 1984). Therefore, this relatively lower peak rate of energy expenditure during late lactation may be associated with the relatively long period of lactation and slow growth rate of the young typified by arboreal mammals and primates (see Chapter 3).

The peak of daily energy expenditure for a 1kg ringtail possum suckling back young (765.5 kJ.day⁻¹) is 21.5% higher than the value predicted by Nagy (1987) for a 1kg marsupial. However, this value is not significantly different being within the 95% confidence limits of the prediction (372 to 1102 kJ.day⁻¹). Thompson and Nicoll (1986) have shown that the maternal basal metabolic rate of a marsupial *M. domestica* is substantially elevated during lactation. Therefore, it may be

TABLE 6.7

Energy and water metabolism during lactation in free-living marsupials

Species	Stage of lactation * ¹	Metabolic rate (%NL female)* ²	Water influx (%NL female)* ²	References
<i>Isodon macrourus</i>	Phase 2a ?	-	+10.0%	Hulbert and Gordon (1972)
	Phase 2b ?	-	+40.0%	
<i>Trichosurus vulpecula</i>	?	-	+14.5%	
<i>Petrogale inornata</i>	?	-	+20.2%	Kennedy and Heinsohn (1974)
<i>Gymnobelideus leadbeateri</i>	Phase 2a	-0.3%	-12.0%	Kennedy and Heinsohn (1974)
<i>Dasyurus viverrinus</i>	Phase 2a		0.9%	Green and Eberhard (1983)
	Phase 3	+83.0%	+59.0%	
<i>Petaurus breviceps</i> * ³	Phase 2a	-1.0%	+33.0%	Nagy and Suckling (1985)
<i>Antechinus swainsonii</i>	Phase 3	+72.0%	+93.0%	Lee and Nagy (1986)
<i>Pseudocheirus peregrinus viverrinus</i>	Phase 2a	-6.0%	+1.5%	This study
	Phase 2b	+19.0%	+3.8%	
	Phase 3	+30.0%	+35.7%	

*¹ Defined in Chapter 2.*² Percentage of non-lactating females metabolic rate or water influx rate.*³ Based on comparison with only one non-lactating female.

? Phase of lactation unspecified.

suggested that the increase in daily metabolic rate for the ringtail possum in this study includes a component of increasing basal metabolic rate. However, preliminary measurements of basal metabolic rate in another marsupial, *Bettongia gaimardi* using respirometry showed no significant differences during lactation (Rose, 1987). In addition, there are problems associated with estimating maternal metabolism using respirometry (Racey and Speakman, 1987) which may result in substantial errors.

6.4.4 Feeding rate

Food requirements of ringtail possums in the wild have not previously been determined. The male and non-breeding females in this study consumed 64 and 59 g dry food.kg⁻¹.d⁻¹ respectively to provide the energy used in oxidative metabolism, which is approximately 20% higher than that predicted for a 1kg marsupial (51 g.kg⁻¹.d⁻¹, 95% CI = 32 to 85 g.kg⁻¹.d⁻¹; Nagy, 1987). The estimated food intake of wild females (non-lactating) is 33% higher than that measured in captive females. Therefore, the ringtail possum increases its intake of foliage to cope with the extra energy demands of free-living. A relatively high estimate of food intake was also obtained for the greater glider *Petauroides volans* (Foley *et al.*, 1989) These authors attributed the high feeding rate to the low digestibility and metabolisability of eucalypt foliage.

The food intake needed to provide the metabolisable energy for females during late lactation was 27% higher than the food intake of non-lactating females and approximately 60% higher than food intake in captivity (see Chapter 5). These food intake estimates for free-living ringtail possums are based on the assumption that the animals were in energy balance. However, females tended to become leaner during Phase 3 of lactation. Furthermore, an increase in foliage intake would involve a faster rate of passage through the gut, which would be accompanied by a fall in the digestibility of dry matter (Chilcott and Hume, 1984a). The mass of the gut increases during lactation in some mammalian species and is assumed to be an adaptive response to allow increased food consumption (Peters and Krynen 1966). Nevertheless, there is no evidence of this occurring in the ringtail possum during lactation. This suggests that females metabolise body fat (deposited during the early stages of lactation) to cope with the added metabolisable energy required during late

lactation, instead of substantially increasing food intake.

The estimated food consumption rates of ringtail possums made in this study are probably lower than the actual rates, since estimates do not include the food that appears as new biomass. Hence, the food ingested by lactating females is underestimated by the amount of energy consumed and transferred directly to the young in the milk and food materials that appear as new biomass (i.e., mammary tissue; fat storage). Females, during the late stage of lactation, when milk production peaks, spent more time feeding than other groups of females (see Chapter 4). This observation probably relates to the extra energy and nutrients required which are transferred directly to the young in the milk. The reproductive strategy in many populations of marsupial folivores including the ringtail possum, ensures that late lactation and weaning of offspring occurs in late spring and early summer when young foliage is most abundant (Pahl and Lee, 1988; see Chapter 3). Young foliage contains more nitrogen and water, but less fibre than mature foliage (Cork and Pahl, 1984; Pahl and Hume, 1988 cited in Pahl and Lee, 1988) and ringtails are known to prefer juvenile leaves (Pahl, 1984). Therefore, by consuming the more digestible young *L.laevigatum* foliage present in the spring the ringtails may increase their rate of food intake without involving a large increase in dry matter intake.

6.4.5 Water metabolism

Information on water flux rates in marsupials has been summarised by Nagy and Peterson (1988). These authors assessed the influence of allometry on water fluxes of animals and compared laboratory and field studies in a variety of taxonomic groups. The water influx rates of a 1kg male and non-lactating female ringtail possum are only 71% of that predicted for a 1kg marsupial ($159 \text{ ml.kg}^{-1}.\text{d}^{-1}$, Nagy and Peterson, 1988). However the water influx rates are not significantly lower than the predicted value because they are within the 95% confidence limits of the prediction (66 to 385 ml/kg, Nagy and Peterson, 1988). A relatively low water influx rate has also been noted for other marsupial arboreal folivores (see Table 6.8). Free-living herbivorous eutherians have higher water flux rates than carnivorous eutherians (Nagy and Peterson, 1988). In contrast, these authors note that herbivorous marsupials show lower water flux rates when compared with

carnivorous marsupials. They suggest that the herbivorous marsupials studied (including the arboreal folivores) consume vegetation which may contain less water than the more succulent vegetation consumed by most eutherian herbivores. This hypothesis is supported by the fact that the leaves eaten by the ringtail possum on Flinders Island had a relatively low water content (50%, see Chapter 4). Similarly, the eucalypt leaves consumed by greater gliders, *Petauroides volans*, had a low water content which probably accounted for the low water influx rate measured for this species (Foley *et al.*, 1989).

The main components of the water influx of ringtail possums were preformed water in the leaves and metabolic water. However, the ringtail possums also ingested free water. Animals in the wild probably obtained free water from dew or rainwater on the leaves, water caught in the twisted trunks of tea-trees and from the fresh water lagoon within the study area. Licking rainwater from fur has also been noted in captive animals. This significant intake of non-food water has also been noted in *Petauroides volans* (Foley *et al.*, 1989) However, it contrasts to that found for the koala, *Phascolarctos cinereus*, which obtains sufficient water from the foliage it consumes to maintain water balance (Nagy and Martin, 1985).

The lack of significant seasonal variation in water turnover by male ringtail possums is similar to that observed for free-living *T. vulpecula* and rock wallabies, *Petrogale inornata* (Kennedy and Heinsohn, 1973). Food water status and food availability are two variables which may influence the rate of water influx in an animal. Variations in water intake in relation to rainfall, food succulence and availability, is most evident in grazing macropodid species (Green, 1989). The water content of grasses can vary considerably, depending on season and locality. However, as expected for sclerophyllous vegetation, there was no seasonal variation in the leaf water content of the major tree species consumed by the ringtail possums on Flinders island (Chapter 4). In addition rainfall was fairly uniform throughout the months studied (Chapter 2). These two factors would contribute to the constancy of water turnover between the seasons noted in male ringtail possums compared with other free-living marsupial species (*Isodon macrourus*, Hulbert and Gordon, 1972; *Gymnobelideus leadbeateri*, Smith *et al.*, 1982; *Dasyurus viverrinus*, Green and Eberhard, 1983; several Macropodid species, reviewed by Green, 1989).

The maximum rates of water influx for ringtail possums were recorded for

females suckling back-young. An increase in rates of water influx during lactation with a significant peak during Phase 3 has also been indicated in studies on other free-living marsupial species (Table 6.7). This increased water requirement reflects increased water effluxes. Water may leave the lactating female via three main pathways (Green and Eberhard, 1983):

- 1) Water lost in the milk sucked from the female by the young.
- 2) Increased evaporative loss associated with metabolic processes involved in the production of milk (formation and secretion)
- 3) Water lost in the process of foraging for food to supply the extra water required for the first two factors.

Water efflux via these routes was obviously greatest in female ringtail possums suckling back young. In contrast, females suckling pouch young showed no significant changes in their rate of water flux. (see Table 6.7). Similarly, captive Tasmanian devils, *Sarcophilus. harrisii* (Nicol, 1978) and *Dasyurus viverrinus* (Green and Eberhard, 1983) showed no significant difference in water intake rates between non-lactating females and those suckling early stage pouch young. Therefore, marsupial young at this stage do not have a measurable effect on the water requirements of the mother. However, a complication in measuring water turnover rates in females which are suckling pouch young is that the calculated rates will be lower if the mother consumes the excreta from the young as she cleans out the pouch (Baverstock and Green, 1975). Nevertheless, this recycling of water as excreta will produce only minor errors because the body water pool size and daily milk intake of the pouch-young are only a small fraction of the mothers body water pool (Dove *et al.*, 1989).

The increased requirement for water during late lactation in the ringtail appears to be predominantly met by an increase in food intake. The apparent increase in intake of drinking water by females during late lactation may be overestimated for two reasons. Firstly these females may feed on young foliage with a higher water content than mature foliage. Secondly, the estimated food water is based on food energy required for oxidative metabolism by the animal and does not take into account the proportion of food consumed which is diverted to milk solids and is not metabolised by the mother.

In summary, the energy and water requirements of adult male ringtail

possums showed no significant seasonal changes, however, those of adult female ringtail possums varied according to their reproductive status. The greatest total daily energy expenditure by any ringtail possum was that of females during Phase 3 of lactation. Total body water estimates suggest that the increased requirement for metabolisable energy during late lactation may be met, in part, by energy stored during the earlier stages of lactation. The maximal rate of energy metabolism by lactating ringtail possums includes the costs of obtaining and digesting additional food for lactation and of milk synthesis, but does not include the energy content of the milk itself which is transferred to, and utilized by, the young. Therefore, before a complete idea can be obtained of the impact of lactation on the energy budget of the female ringtail possum, the amount of energy transferred to the young in milk must be estimated.

CHAPTER 7

MILK COMPOSITION IN THE COMMON RINGTAIL POSSUM

7.1 Introduction

Lactation is a unique characteristic of mammals, playing an important role in the evolution of mammalian patterns of reproduction (Maynard-Smith, 1977; Pond, 1977; Daly, 1979; Oftedal, 1980). Despite their separate evolution, marsupials, monotremes and eutherians show similarities in their mammary gland structure and its cellular development, all exhibiting a lobulo-alveolar secretory structure (Tyndale-Biscoe and Renfree, 1987). Differentiation of the mammary gland in marsupials occurs from specialised epidermal cells which form mammary hair follicles with associated glands (Bresslau, 1920). Each hair follicle gives rise to a mammary duct or galactophore. Before puberty the mammary duct exists without alveoli and the teats are inverted. When the animal reaches sexual maturity the mammary hairs are shed and the teats become everted (Bresslau, 1920). These special mammary hairs are peculiar to marsupials and monotremes and are not found in the developing mammary gland of eutherians (Tyndale-Biscoe and Renfree, 1987).

After parturition the glands to which the marsupial young attach become enlarged and begin to lactate. The glands continue growing throughout lactation as does the associated teat (Tyndale-Biscoe and Renfree, 1987). These changes have been described in several marsupial species including; the eastern quoll, *Dasyurus viverrinus* (O'Donoghue, 1911), the American opossum, *Didelphis virginiana* (Hartmann, 1923), the brushtail possum, *Trichosurus vulpecula* (Sharman, 1962), the red kangaroo, *Macropus rufus* (Griffiths *et al.*, 1973), the agile wallaby, *Macropus agilis* (Lincoln and Renfree, 1981), the tammar wallaby, *Macropus eugenii* (Findlay, 1982; Stewart, 1984) and the potoroo, *Potorous tridactylus* (Crowley, 1984).

Studies on lactation in eutherian mammals are extensive and cover such aspects as composition of the milk (reviewed by Jenness and Sloan, 1970 and Oftedal, 1984), the bioenergetics of milk production and growth (Oftedal, 1984, 1985) and the physiology and biochemistry of lactogenesis, particularly in domestic

species (see Kon and Cowie, 1961; Larson and Smith, 1974; Peaker, 1977). However, information on lactation in marsupials is less abundant.

The milks of marsupial species studied to date show major compositional changes throughout lactation, both quantitative and qualitative, but with little variation between species (Green, 1984; Green and Merchant, 1988). This is in contrast to the milks of eutherian species which show less substantial compositional changes over lactation, but great variation between species (Ofstedal, 1984). Quantitative studies have been made of proximate milk constituents (i.e., protein, lipid, carbohydrate and water) in a range of marsupial species; the wallaroo, *Macropus robustus* (Bolliger and Pascoe, 1953), *Trichosurus vulpecula* (Gross and Bolliger, 1959), *Didelphis virginiana* (Bergman and Housley, 1968), *Macropus rufus* (Lemon and Barker, 1967; Poole *et al.*, 1982), the grey kangaroos, *Macropus giganteus* and *Macropus fuliginosus* (Poole *et al.*, 1982), *Dasyurus viverrinus* (Green *et al.*, 1987), the numbat, *Myrmecobius fasciatus* (Griffiths *et al.*, 1988), *Potorous tridactylus* (Crowley *et al.*, 1988); the bettong, *Bettongia gaimardii* (Smolenski and Rose, 1988), the northern brown bandicoot, *Isodon macrourus* (Merchant and Libke, 1988) and the red-necked wallaby, *Macropus rufogriseus banksianus* (Merchant *et al.*, 1989). These studies reveal an overall common pattern of change in the concentration of milk components

Qualitative studies of milk components have also been made in a number of species; *Trichosurus vulpecula* (Gross and Bolliger, 1958); *Macropus rufus* (Lemon and Bailey, 1966, McKenzie *et al.*, 1983); the quokka, *Setonix brachyurus* (Jordan and Morgan, 1968); *Macropus eugenii* (Messer and Green, 1979, Green *et al.*, 1983, Green and Renfree, 1982, Nicholas *et al.*, 1987); *Macropus giganteus* (McKenzie *et al.*, 1983); *Dasyurus viverrinus* (Green *et al.*, 1987). These studies show that major changes in the types of carbohydrates, amino acids and fatty acids of the major milk constituents occur throughout lactation.

In all mammals the reproductive female must acquire, process and transfer sufficient nutrients to her young to support growth up to weaning (Gittleman and Ofstedal, 1987). However, the composition of the milk of a particular species may not be influenced only by the nutritive requirements of the young. Ben Shaul (1962) was the first to suggest a general correlation between milk composition and the nursing habits of a species. Mammals that suckle their young on a demand basis

appear to produce dilute milk with a low fat content (e.g. primates) compared to those that suckle their young infrequently (e.g. otariid pinnipeds and Lagomorpha). The influence of behavioural, dietary and ecological factors on the milk composition of species have been further noted by Jenness and Sloan (1970) and Oftedal (1984). More recently, Gittleman and Oftedal (1987) suggest that growth rates of species may reflect constraints on nutrient transfer imposed by maternal diet and type of parental care, based on their comparison of growth and lactation in carnivores

Although the studies of milk composition in marsupial species in recent years suggest that the lactational pattern is basically the same, there are minor differences between species, for example, in the times of appearance and the maximum concentrations of the various components (Merchant *et al.*, 1989). The data available for Macropodoidea suggest that peak concentrations of milk solids may be inversely related to body mass (Merchant *et al.*, 1989). Furthermore studies of milk composition in marsupials of different trophic groups, i.e; a carnivorous species (Green *et al.*, 1987) an omnivorous species (Merchant and Libke, 1988) and herbivorous species (Lemon and Barker, 1967; Messer and Green, 1979; Poole *et al.*, 1982; Green and Renfree, 1982; Green *et al.*, 1983; Smolenski and Rose, 1988; Crowley *et al.*, 1988), suggest that lactation may be adapted to the lifestyle of a particular species (Merchant and Libke, 1988; Merchant *et al.*, 1989). Lactation in folivorous marsupials is of particular interest since these species are characterized by small litter sizes, slow growth of the young, a long period of lactation and low rates of offspring production compared with other marsupials of a similar size (Smith and Lee, 1984). However, milk composition has been examined in detail in only one folivorous species, *Trichosurus vulpecula* (Cowan, 1989). The common ringtail possum is the smallest folivorous arboreal marsupial and a study of lactation in this species may reveal whether it shows any major differences from the general marsupial pattern of lactation which may reflect the influence of the lifestyle of this species.

The aspects of lactation examined in this chapter are, quantitative and qualitative milk composition, and growth of the mammary gland. The metabolisable energy expenditure of lactating ringtail possums has been examined in the previous chapter. This study will examine the composition of the milk at all stages of lactation which is the first step in the determination of the transfer of nutrients and energy from the mother to her young, crucial to the understanding of the total

energy requirements for lactation. The growth of the ringtail possum mammary gland has been examined to determine whether or not its growth is correlated with changes in milk composition and production as noted in other marsupial species (Tyndale-Biscoe and Renfree, 1987). The qualitative and quantitative milk composition of the ringtail possum will be compared with that of other marsupial species, and an assessment made of the particular lactational strategy employed.

7.2 Methods

7.2.1 Animals

Milk samples were collected from 8 lactating ringtail possums which were maintained in captivity. Five of these were caught with pouch young on Flinders Island, in areas outside the study areas described in Chapter 2. Within a few days of captivity four of these ringtails had lost their young but mated and gave birth again later. The remaining animal (F2) successfully reared her young (S and T), which had been born in the wild. Details of milk sampling from captive females are summarised in Table 7.1.

Milk samples were also collected from wild animals at Whitemark Beach and Paddies (Chapter 2) on Flinders Island. Eleven lactating females were sampled in 1986 and nine lactating females in 1987. Field milk samples were compared with milk collected from captive animals.

7.2.2 Milk Collection

Milk samples were collected from the captive females when the young had released the teat; between 4 to 7 weeks after birth. Milk was not collected before 4 weeks post-partum for two reasons. Firstly, there was some danger that the young would not re-attach to the teat after milking. Secondly, the difficulty in milking the small gland, experienced at 4 weeks post-partum, suggested attempts to milk at an earlier stage of lactation would have resulted in only minute samples of milk (if any). It was obviously important to ensure that the female kept her young so that sequential milk samples from each animal could be collected.

Prior to milking, the mother was restrained in a canvas bag while the pouch

TABLE 7.1 Milk sample details for captive females.

FEMALE	No of sequential milk samples	Year	Young born in captive or field	No of young
F1	15	1986	captive	2
F1	15	1987	captive	2
F2	8	1986	field	2
F3	12	1986	captive	1
F4	13	1986	captive	2
F4	16	1987	captive	2
F5	11	1986	captive	2
F5	15	1987	captive	2
F6	6	1987	captive	2
F7	17	1987	captive	1
A	2	1987	captive	2

was opened and the young removed from the teats. The young were then placed in labelled containers and maintained in a humid incubator at 35°C in a quiet room. From about 17 weeks of age, when the young had left the pouch, they were kept warm in small calico bags. At each milking the young were weighed to the nearest 0.1g on a Mettler PE 3600 balance. The mother was weighed to the nearest 10g using a Pesola spring balance.

Oftedal (1980) has outlined some important considerations relating to milk sampling and analysis in comparative studies of lactation. The method of milk collection may influence composition especially in regard to fat content. The degree of mammary emptying is important; studies on eutherian mammals have shown that the initial milk obtained at a milking is lower in fat than milk obtained at the end of mammary gland stripping. In wild animals, handling stress will increase circulating levels of adrenalin, which inhibit the endogenous secretion of oxytocin and thus reduce the milk ejection reflex. A combination of dam/young separation, sedation and exogenous oxytocin injection should facilitate milk collection and minimize sampling bias (Oftedal, 1980).

In this study, milk was allowed to accumulate in the mammary glands for between two and six hours, depending on the age of the young. A few drops of Fluothane anaesthetic (Halothane B.P, ICI) were placed on cotton wool in a glass jar and the mother was then anaesthetised by holding the jar over her nose. Immediately prior to milking an intramuscular injection of 0.1ml oxytocin (10IU/ml Syntocinon; Sandoz, Australia) was administered. The teats and surface of the mammary gland were wiped clean with a paper tissue. When the milk appeared at the tips of the teats it was collected in one of three different ways depending upon the stage of lactation.

During early lactation the small amounts of milk that were extruded from the teat by gently massaging the gland, were collected in non-heparinized microhaematocrit tubes. These were flame sealed, labelled and then stored at -20°C until analysed. During the middle and late stages of lactation, milk was collected using a syringe device (as in Crowley, 1985 and Smolenski, 1986) or directly from the end of the teat into a 1 or 2ml plastic vial. The mammary gland was massaged gently in both of these methods as this aided milk flow. Whenever possible milk samples collected from the individual mammary glands belonging to the same female were kept separate and labelled according to the position of the mammary gland, either left or right. All milk samples were labelled, sealed with laboratory

film (Parafilm "M", American Can Company) and then frozen at -20°C . In order to minimise fat deterioration by lipase activity and, to a lesser extent protein breakdown (Woodward pers. comm.), all analyses were carried out within 9 months of milk collection.

Attempts at milk collection without the use of oxytocin or anaesthetic resulted in insufficient quantities of milk being obtained for analysis. Using the above techniques between 0.5ml and 1.5ml of milk was collected depending upon the individual animal and the stage of lactation. Milk was collected from the captive females at intervals of between 7 and 14 days. After the milking, the young were returned to their mother by replacing them in the pouch next to the teat from which they had been removed. It was found that when placed next to the teat, the pouch young were able to easily grasp it without assistance

Milk samples from lactating females caught in the wild were collected using the same techniques. Only females whose young had well developed pelage were milked. This prevented the possible loss of pouch young from the study population due to the difficulty of keeping the hairless young warm and moist under field conditions. Field milk samples were labelled, sealed with laboratory film and then placed in a cool container and transported to the laboratory where they were frozen at -20°C . Prior to analysis, all milk samples were thawed and homogenized by shaking.

7.2.3 Mammary Gland

The mammary gland was measured at each milking along the two longest axes at right angles and an index of size calculated by multiplying these two measurements (see Fig. 7.1). The mammary gland index illustrates changes in the size of the gland without involved calculations or complicated measurements (Findlay, 1982; Green, 1984; Green *et al.*, 1988). It is a "surface area" measurement which correlates, for the different stages of lactation, with the actual weight measurements made in *Trichosurus vulpecula* (Smith *et al.*, 1969) and *Macropus eugenii* (Tyndale-Biscoe *et al.*, 1984). The right mammary gland was always measured, using vernier calipers, except where the individual female only had one young and the left mammary gland was the functional gland.

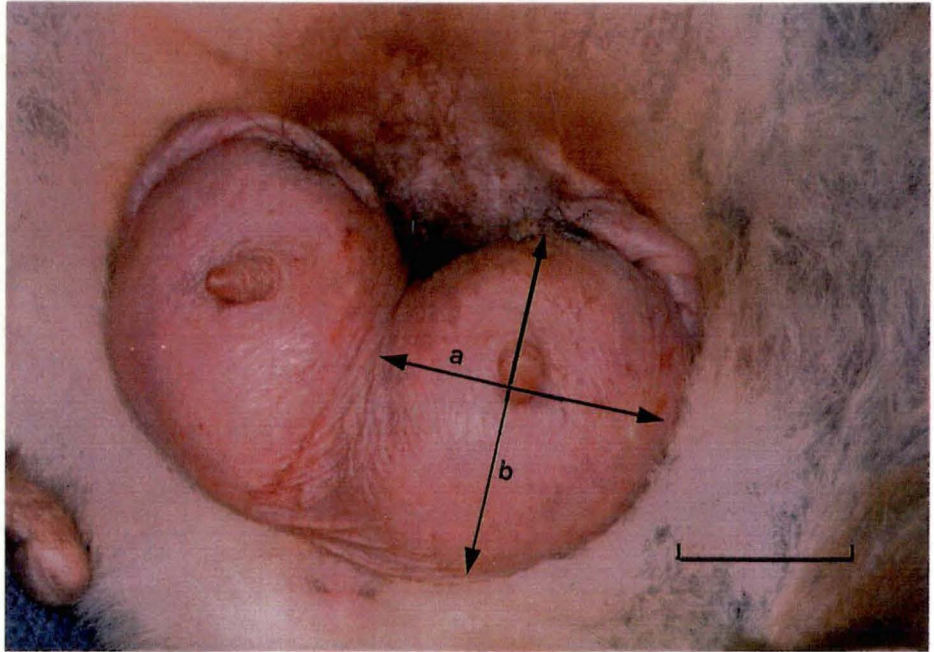


Figure 7.1 Mammary glands of a ringtail possum suckling two 26 week old young. Measurements made for Mammary Index are illustrated (a x b). Scale line = 1cm

7.2.4 Milk Composition

Total Solids

Depending on the availability of milk, volumes of between 50 μ l and 80 μ l were weighed in pre-weighed plastic vials. Each sample was then freeze-dried, or oven-dried at 100°C for 24 hrs and then re-weighed. All weighings were made to the nearest 0.0001g on a Mettler AE 100 balance. The ratio of dry mass to wet mass provided a relative estimate (%) of total solids. When only small volumes of milk were available, as with early milk samples and some field samples, 20 μ l to 40 μ l were weighed on pre-weighed foil to the nearest 1 μ g with a Cahn Electrobalance. Each sample was then oven dried at 100°C for 24 hrs, cooled in a desiccator and re-weighed.

Carbohydrate

The carbohydrate content of the milk was determined as total hexose using the phenol-sulphuric acid method (Dubois *et al.*, 1956) as modified by Messer and Green (1979). In this procedure 5 μ l of milk were diluted with 2mls of distilled water and then 200 μ l aliquots were mixed with 1ml of 3.55 % (w/v) phenol solution in a test tube. Concentrated sulphuric acid (3 mls) was then added rapidly, the stream being directed against the liquid surface to obtain good mixing and maximum generation of heat. The solution was then allowed to cool for 30 minutes after which the absorbance was measured at 490 nm in a Shimadzu UV-120 spectrophotometer. The standard used to prepare a standard curve for each assay was 0.0305 % lactose solution.

Samples of whole milk from captive ringtails were prepared for thin-layer chromatography by Dr. M. Messer Biochemistry department, University of Sydney, N.S.W. to monitor the qualitative changes in milk carbohydrate.

Protein

The total protein content of the milk was determined using the Protein-Dye Binding method of Bradford (1976). Initially the protein reagent was prepared by dissolving 100mg of Coomassie Brilliant Blue G250 (Eastman Kodak Co., Rochester, N.Y., U.S.A) in 50ml of 95% ethanol. 100 mls of 85% (w/v) phosphoric acid were added to this solution. The resulting solution was diluted to 1 litre with distilled water, filtered and stored at room temperature. It was found that

this solution was stable for up to three weeks.

A protein standard was prepared by dissolving 25mg of bovine serum albumin in 25ml of 0.9% saline (1mg/ml). This solution was diluted (1 : 5) in distilled water to provide a working solution of 200µg/ml. For the assay of the samples, 5µl of whole milk were diluted into 2ml distilled water and 200µl aliquots pipetted into test tubes. 3 mls of protein reagent were added to the samples and standards. The contents of the test tubes were mixed by inversion and vortexing. Between five and twenty minutes later the absorbance was read at 595nm using a UV-120 Shimadzu spectrophotometer.

Whey proteins in whole milk samples collected from captive ringtail possums were examined by SDS polyacrylamide-gel electrophoresis (SDS PAGE) at CSIRO, Division of Wildlife and Ecology, Canberra, A.C.T by Dr. K. Nicholas and M. Loughnan. Individual whey proteins were isolated and purified according to the method described by Nicholas *et al.*, 1989 (see Appendix F). The timing and appearance of these individual whey proteins, in particular the lysozyme and α-lactalbumin fractions, were noted.

Lipid

The fat content of milk was estimated using the creatocrit method described by Lucas *et al.* (1978). This method was developed to provide a quick and simple technique to measure fat in small quantities of human milk. The technique has been tested for accuracy by comparison with gravimetric and triglyceride analytic methods (Hudson *et al.*, 1979) and has been particularly useful in the estimation of the fat content of marsupial milk when only small quantities are available (Crowley *et al.*, 1988; Smolenski and Rose, 1988). In the present study 50µl - 75µl of well-mixed milk was collected into a standard glass capillary tube. One end of the tube was flame sealed and the tube was then spun in a Hawksley Haematocrit centrifuge for 15 minutes at maximum speed. The percentage creatocrit was calculated as:

$$\frac{\text{length of cream and liquid fat layer}}{\text{total length of milk column}} \times 100$$

After spinning whole milk the cream layer appeared as a separate opaque layer with a thin layer of clear liquid fat above. The cream layer and the liquid fat layer together represent the lipid fraction of the milk so the liquid fat was included in the measurement (Woodward, pers. comm.). Measurements were taken to the top of the meniscus within 30 minutes to avoid errors caused by spreading of the cream layer. The method was standardised using the Roesse-Gottlieb ether extraction technique (cited in Horwitz, 1980). A linear relationship between the creamatocrit method and the extraction method was derived from which grams of lipid per 100ml of milk could be estimated as;

$$y = -0.4093 + 0.4452x \quad (r^2 = 0.90, n = 24) \text{ (Fig. 7.2).}$$

Where 'y' is the Roesse-Gottlieb measure of lipid (g/100ml) and 'x' is the % creamatocrit. Field milk samples were included in this regression.

Milk Electrolytes

Estimates of sodium, potassium, calcium and magnesium content were made by diluting 5µl of milk to 2ml with de-ionised water and then analysing the diluent by atomic absorption spectrophotometry (Varian, 1000).

7.2.5 Statistical Methods

Differences between means were tested by two-tailed t-tests and regression lines were calculated using the least squares linear regression method. A two-way analysis of variance (ANOVA) with unequal sample size was used to compare milk component concentration between years (Sokal and Rohlf 1969; BIOSAT). It was assumed that there were no inter-animal differences, and hence repeated samples from an animal were treated as independent samples. However, it is acknowledged that possible inter-animal differences may bias the results of statistical comparisons. In all tests the 5% level of probability was accepted as indicating statistical significance.

7.3 Results

7.3.1 Mammary Gland Size and Pouch Condition

Immediately prior to birth the posterior mammary glands were inconspicuous, but the teats were erect and bulbous. The anterior mammary glands were also inconspicuous but the teats were inverted in all of the females that were studied.

The mammary gland was measured initially at week 5 of lactation, when the

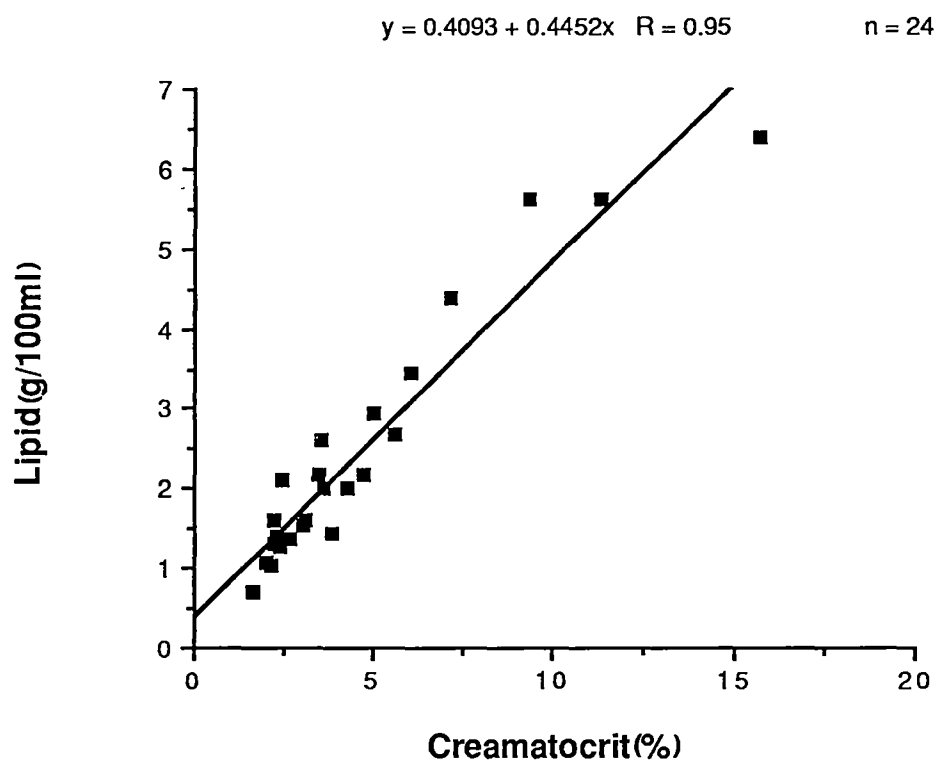


Figure 7.2 Linear regression of values obtained using the Creamatocrit and Roese-Gottlieb methods of fat analysis.

young first released the teat. From week 5 to week 12 of lactation the increase in size of the gland was relatively small, however elongation of the teats was noted (Figure 7.3a). From week 12 to week 16 the gland grew rapidly and the mammary index doubled. After permanent emergence of the young from the pouch, the mammary gland steadily decreased in size. This occurred at a time when the young were eating increasing amounts of solid food and probably sucking less frequently. By the time the young were fully weaned at week 30 of lactation the mammary gland had regressed completely and was no longer secreting milk. Thus the sucked mammary gland increased substantially in size during Phase 2 of lactation, but decreased in size after pouch emergence (Fig 7.4)

Immediately prior to birth the degree of invagination of the pouch was estimated to be between 1.5cm and 2cm. An oily red/brown secretion was observed covering the pouch lining, surrounding the teats and around the pouch entrance. The pouch entrance at this stage was constricted. Between 16 and 18 weeks of lactation the pouch entrance had relaxed and after the young had left the pouch the mammary glands were often observed to be completely exposed (Figure 7.3b). The red/brown secretion lining the pouch gradually disappeared at this stage.

The single active mammary gland of a female which suckled only one young showed no difference in its size when compared with individual glands of females suckling two young. One female (F4) reared two young up to week 10 of lactation, and then a single surviving young to weaning. The single remaining active mammary gland of this female increased in size threefold (Figure 7.3c) whilst the non-sucked gland regressed rapidly to its quiescent state.

7.3.2 Milk Composition

The milk composition data for females which suckled pouch young in 1986 and for those which suckled young in 1987 were compared using a two-way ANOVA, with unequal sample sizes, for each constituent (Table 7.2). Total solids, carbohydrate, fat, sodium and potassium showed no significant difference between years, so the data for these milk constituents were combined. However, the protein values were significantly different between years ($F_{1,45} = 6.93$, $P < 0.05$) and therefore the data are presented separately.

Figure 7.3

- (a) Ringtail pouch at 9 weeks of lactation illustrating elongated teats and tight entrance.



- (b) Ringtail mammary glands at 23 weeks of lactation.



- (c) Single mammary gland at 24 weeks of lactation.



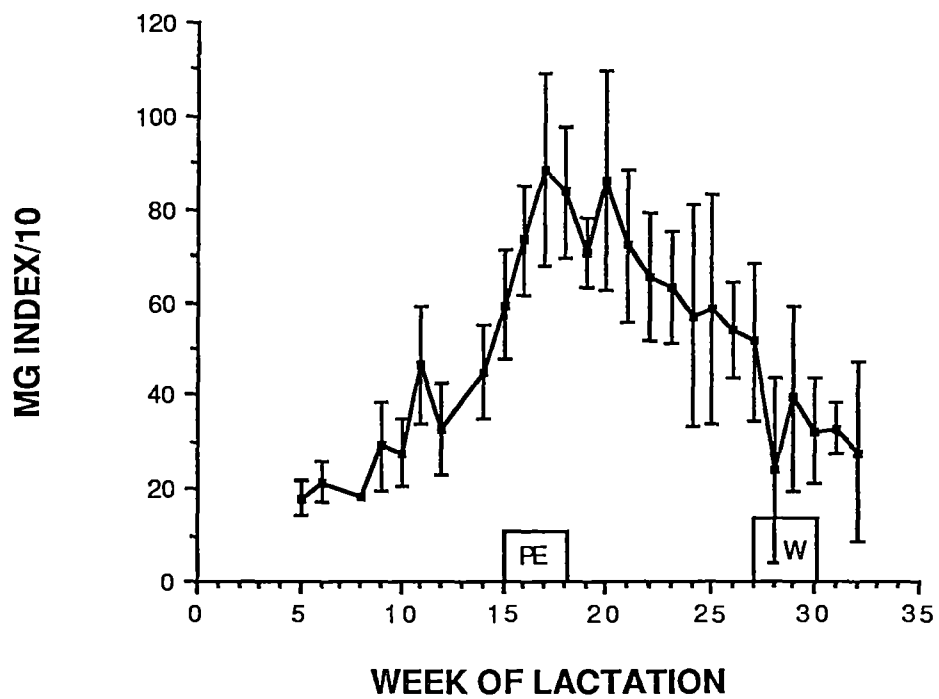


Figure 7.4 Changes in mammary gland size (Mammary Index) during lactation (mean \pm s.d. $n = 113$).
PE = pouch emergence, W = final weaning (see Chapter 3)

TABLE 7.2 Comparison of milk components collected from captive animals in 1986 and 1987 (2-way ANOVA).

<u>SOLIDS</u>	SOURCE	DF	F-RATIO	P
	year	1	3.529	>0.05
	week	8	30.185	<0.05
	year/week	8	1.501	>0.05
<u>CARB</u>	SOURCE	DF	F-RATIO	P
	year	1	3.565	>0.05
	week	6	66.730	<0.05
	year/week	6	0.591	>0.05
<u>PROTEIN</u>	SOURCE	DF	F-RATIO	P
	year	1	6.929	<0.05
	week	6	9.638	<0.05
	year/week	6	0.993	>0.05
<u>FAT</u>	SOURCE	DF	F-RATIO	P
	year	1	1.049	>0.05
	week	6	5.627	<0.05
	year/week	6	1.1513	>0.05
<u>K</u>	SOURCE	DF	F-RATIO	P
	year	1	3.209	>0.05
	week	5	0.965	>0.05
	year/week	5	0.526	>0.05
<u>Na</u>	SOURCE	DF	F-RATIO	P
	year	1	2.932	>0.05
	week	5	4.378	<0.05
	year/week	5	1.657	>0.05

Milk Solids

Between five weeks and ten weeks post-partum, the solids fraction represented about 16% (w/w) (Figure 7.5). By week 14 of lactation, immediately prior to the young emerging from the pouch, the solid material had risen to around 25% (w/w). During the period of temporary pouch emergence (week 15 to week 18 of lactation), the milk solids fraction dropped rapidly to approximately 13% (w/w). The milk remained dilute throughout the remainder of lactation, a time when the young were eating increasing amounts of solid material. Before the young were fully weaned there was a slight rise in solids but this was very variable. This fluctuation probably reflects variation in time to weaning, some young being fully weaned by week 27 and others not until week 30 (see Chapter 3).

The solids fraction of individual milk samples collected from ringtail possums in the wild is also shown in Figure 7.5. The concentration of solids in Phase 3 milk from field animals appears to follow the same pattern as that found for captive ringtails. There was no significant difference between the percentage of milk solids in captive and field milk during Phase 3 of lactation ($df=106$, $t = -0.26$, $P>0.05$; Table 7.3).

Protein

Although the values obtained for protein in 1986 and 1987 were statistically different (Table 7.2), it can be seen from Figure 7.6 that the trends are similar. Samples collected during 1987/88 show that at week 5 of lactation the whole milk protein was 4.40 g/100ml. Between week 5 of lactation and week 14 milk protein remained fairly constant at about 4.50 g/100ml. Milk protein concentrations peaked at week 15 to 8.40 g/100ml before dropping to 6.13 g/100ml at week 16. During late lactation milk protein fluctuated slightly but remained relatively constant at about 5.50 g/100ml before rising rapidly at the end of lactation. The 1986/87 milk samples showed a similar change in milk protein concentration throughout lactation except for the peak at week 15. This peak was not so pronounced in the 1986/87 samples.

The levels of protein (g/100ml) in individual milk samples collected from ringtails in the wild are shown in Fig. 7.7a. For ease of comparison the values obtained for the level of protein in milk from captive animals for 1986/87 and 1987/88 have been combined. The values obtained for protein in Phase 3 milk samples collected from wild animals appear to follow the same trend as those of the

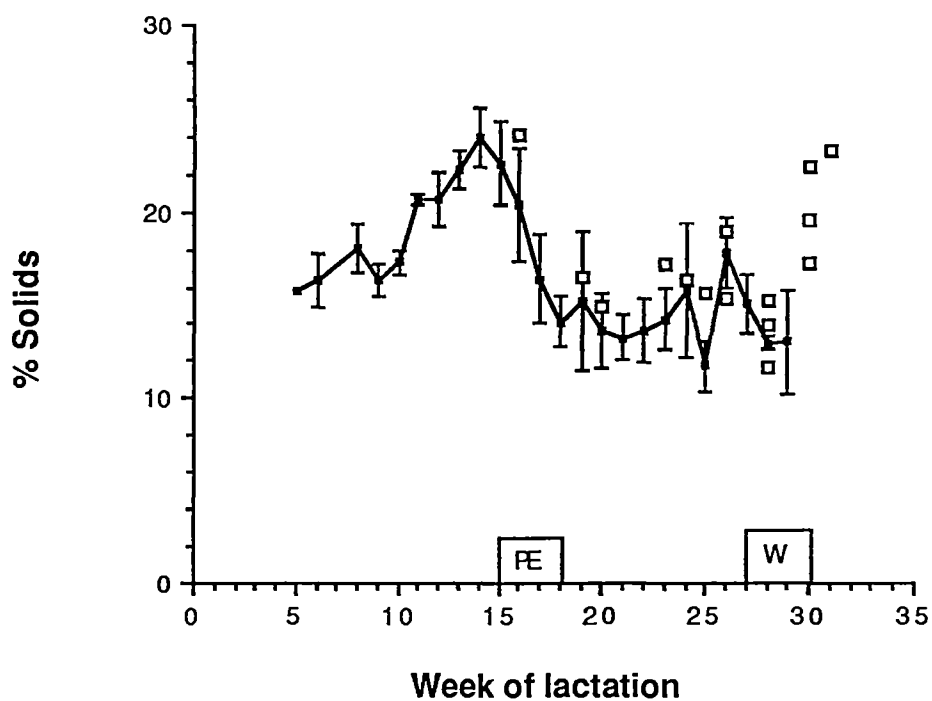


Figure 7.5 Levels of total milk solids in individual milk samples collected from wild *P.peregrinus*, \square ($n = 15$) compared with total milk solids from captive *P.peregrinus*, \blacksquare (means \pm s.d., $n = 122$). PE = Pouch emergence, W = Final weaning.

TABLE 7.3 Comparison of Phase 3 milk components from field and captive animals .

Milk Constituent	Captive	Wild	Un-paired t-TEST (2-tailed)	
	\bar{x} (\pm s.d, n)	\bar{x} (\pm s.d, n)	t-value	P
Solids (%)	16.47 (\pm 4.6, 91)	16.78 (\pm 3.8, 17)	-0.257	>0.05
Carbohydrate (g/100ml)	6.24 (\pm 2.3, 91)	6.00 (\pm 1.3, 17)	0.417	>0.05
Protein (g/100ml)	6.85 (\pm 2.7, 91)	5.01 (\pm 1.2, 17)	2.766	<0.05
Lipid (g/100ml)	2.31 (\pm 1.3, 91)	5.37 (\pm 2.4, 17)	-7.564	<0.05
Na (mMol)	21.45 (\pm 9.3, 70)	22.22 (\pm 6.8, 18)	0.328	>0.05
K (mMol)	20.67 (\pm 4.9, 86)	17.28 (\pm 3.6, 18)	2.764	<0.05
Mg (mg/l)	126.94 (\pm 54.2, 34)	103.57 (\pm 12.5, 7)	-1.125	>0.05
Ca (g/l)	1.64 (\pm 0.5, 33)	1.27 (\pm 0.2, 7)	1.796	>0.05

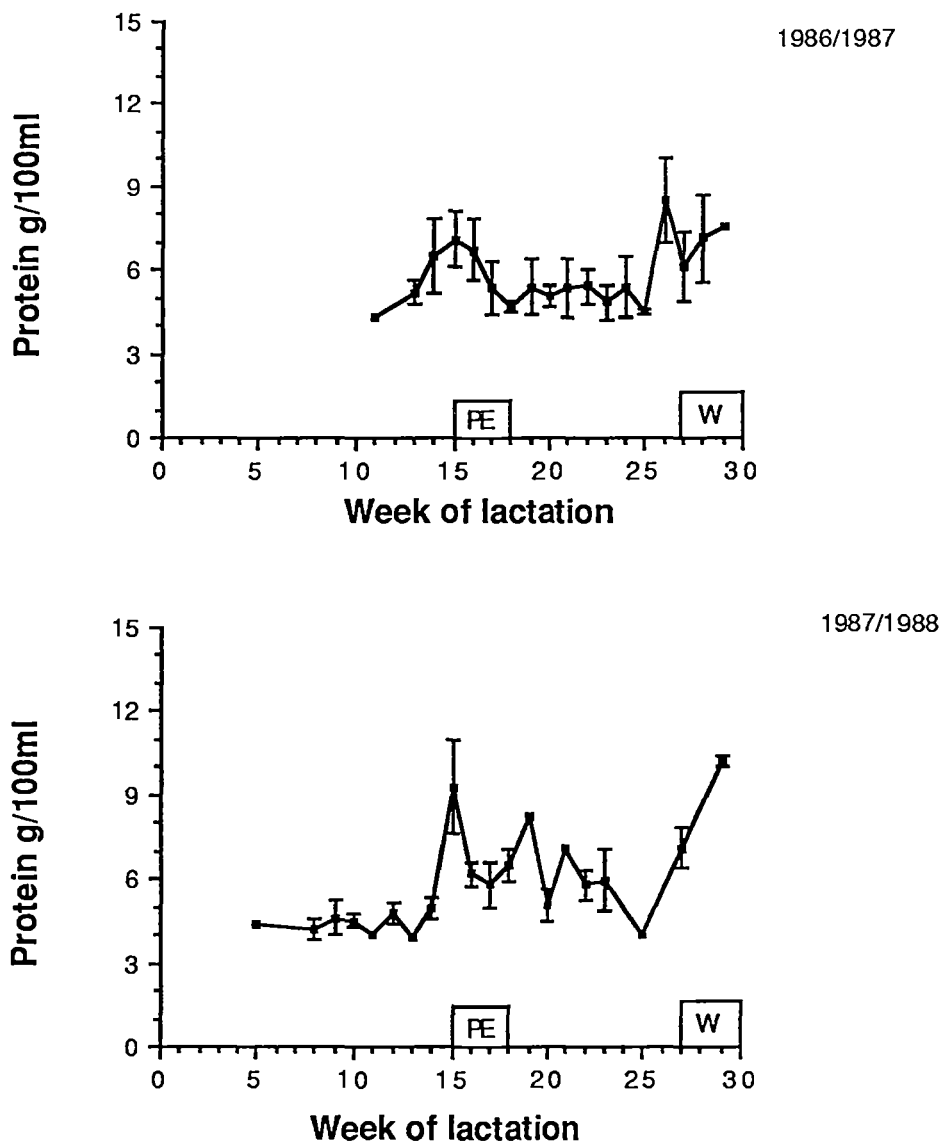
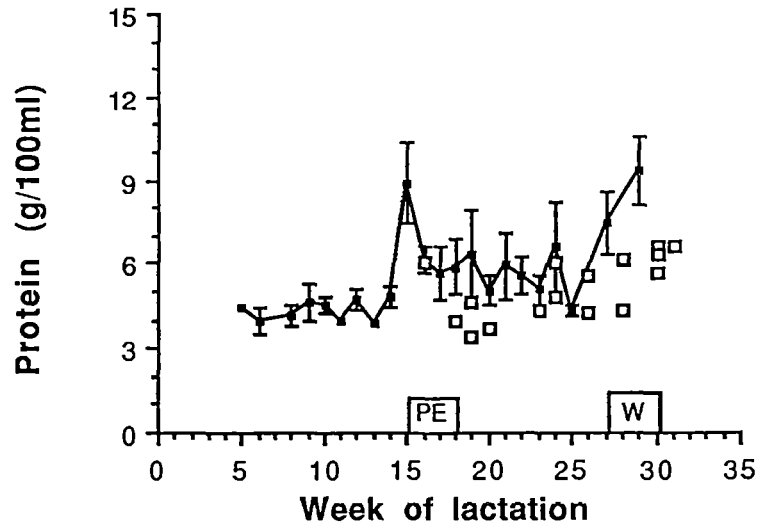


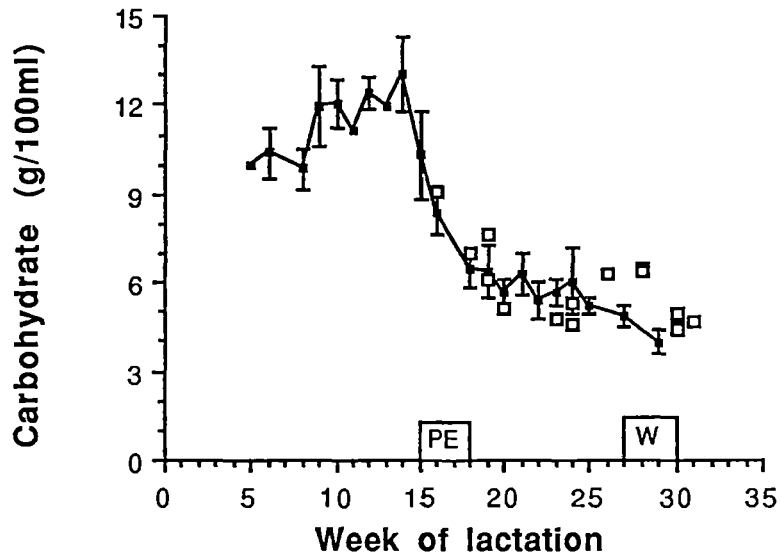
Figure 7.6 Changes in protein concentrations during lactation. Values are given as means \pm s.d.
n (1986/87) = 57, n (1987/88) = 79.
PE = Pouch emergence, W = final weaning.

Figure 7.7 Levels of **(a)** protein (n = 16),
(b) hexose (n = 16),
(c) lipid (n = 15),
in individual milk samples from wild ringtail
possums (□) compared with milk from captive ringtail
possums (■).
(means \pm s.d., n = 122 for each milk constituent).
PE = Pouch emergence, W = Final weaning.

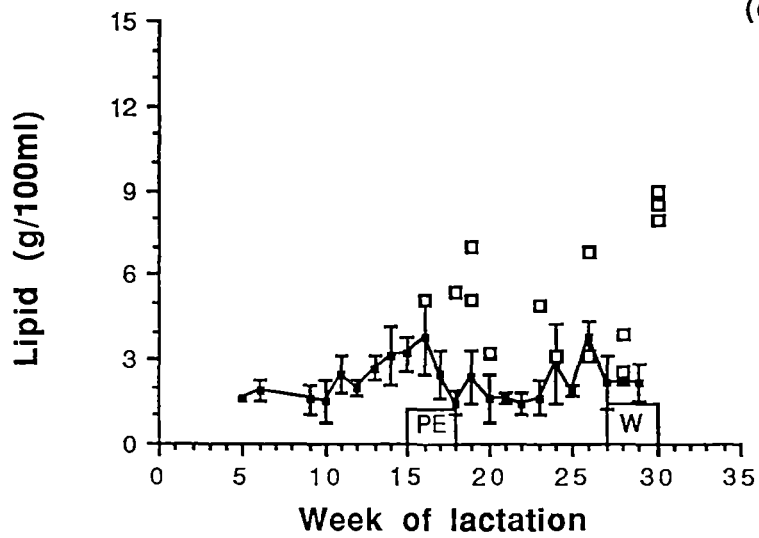
(a)



(b)



(c)



captive milk samples. However, an un-paired t-test showed that field milk protein levels during Phase 3 of lactation were significantly lower than Phase 3 milk from captive animals ($df = 106$, $t = 2.76$, $P < 0.05$; Table 7.3) .

Electrophoresis of the whey proteins in milk samples collected from captive animals reveals changes in milk proteins from 14 to 27 weeks post-partum (Fig. 7.8). The two proteins which run with the low molecular weights were identified as lysozyme and α -lactalbumin. Lysozyme and α -lactalbumin electrophoresed with apparent molecular weights of approximately 14,600 KD and 13,400 KD respectively. α -lactalbumin was present in all milk samples from 14 to 27 weeks but lysozyme appeared between week 16 and 20. At this time a relatively slow moving protein increased dramatically in concentration; this protein was assumed to be transferrin because of its molecular size and because its appearance coincided with a pink coloration of the milk. Serum albumin was present in all milk samples.

Carbohydrates

The total hexose content of milk from captive females is shown in Figure 7.7b. A single sample, obtained in week 5 of lactation, had a hexose content of 10 g/100ml. Between week 5 and week 14 of lactation there was a gradual increase in the total hexose content of the milk to a maximum value of 13 g/100ml. After this there was a rapid decline until week 18 when the young emerged from the pouch. From week 18 until near the time that the young were fully weaned the total hexose content of the milk remained relatively constant at about 6 g/100ml dropping slightly towards the end of lactation to 4 g/100ml.

Figure 7.7b also illustrates the levels of hexose (g/100ml) in individual milk samples collected from ringtails in the wild. As with the milk solids the values obtained for carbohydrate levels in Phase 3 milk appear to follow the same trend as that shown by the captive milk samples. There was no significant difference between carbohydrate levels during Phase 3 of lactation in wild and captive milk ($df = 106$, $t = 0.42$, $P > 0.05$; Table 7.3).

Thin layer chromatography of whole milk samples run against a mixture of standards reveal many components in the milk carbohydrate fraction (Figure 7.9). Between 6 and 14 weeks post-partum, which corresponds with Phase 2b of lactation the milk carbohydrates consisted mainly of oligosaccharides. These oligosaccharides had the same chromatographic mobilities as the standards

Figure 7.8 SDS Polyacrylamide gel (15%) electrophoresis of whey protein from 98 to 187 days of lactation. The position of transferrin (TFN), serum albumin (S.ALB), lysozyme (LYZ) and α - lactalbumin (α -lac) are indicated by the arrows.

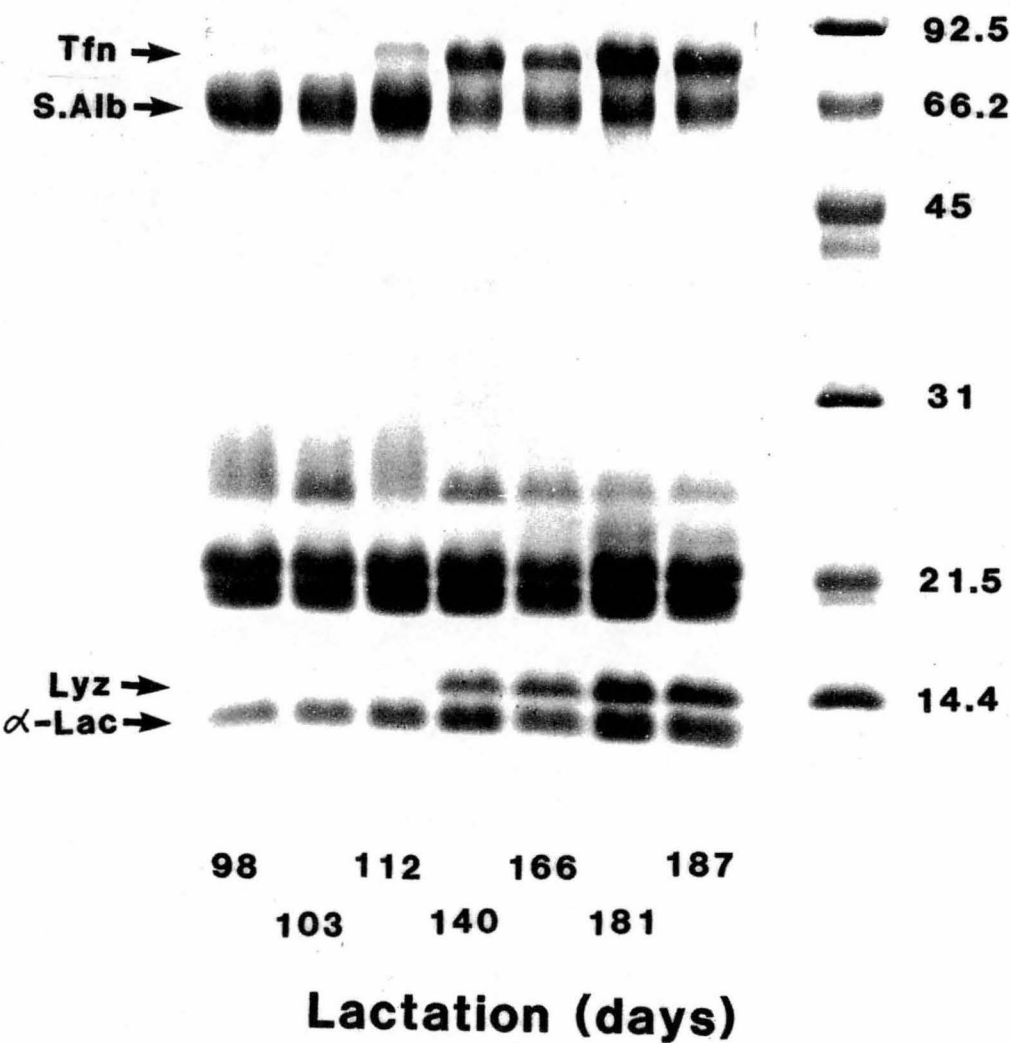


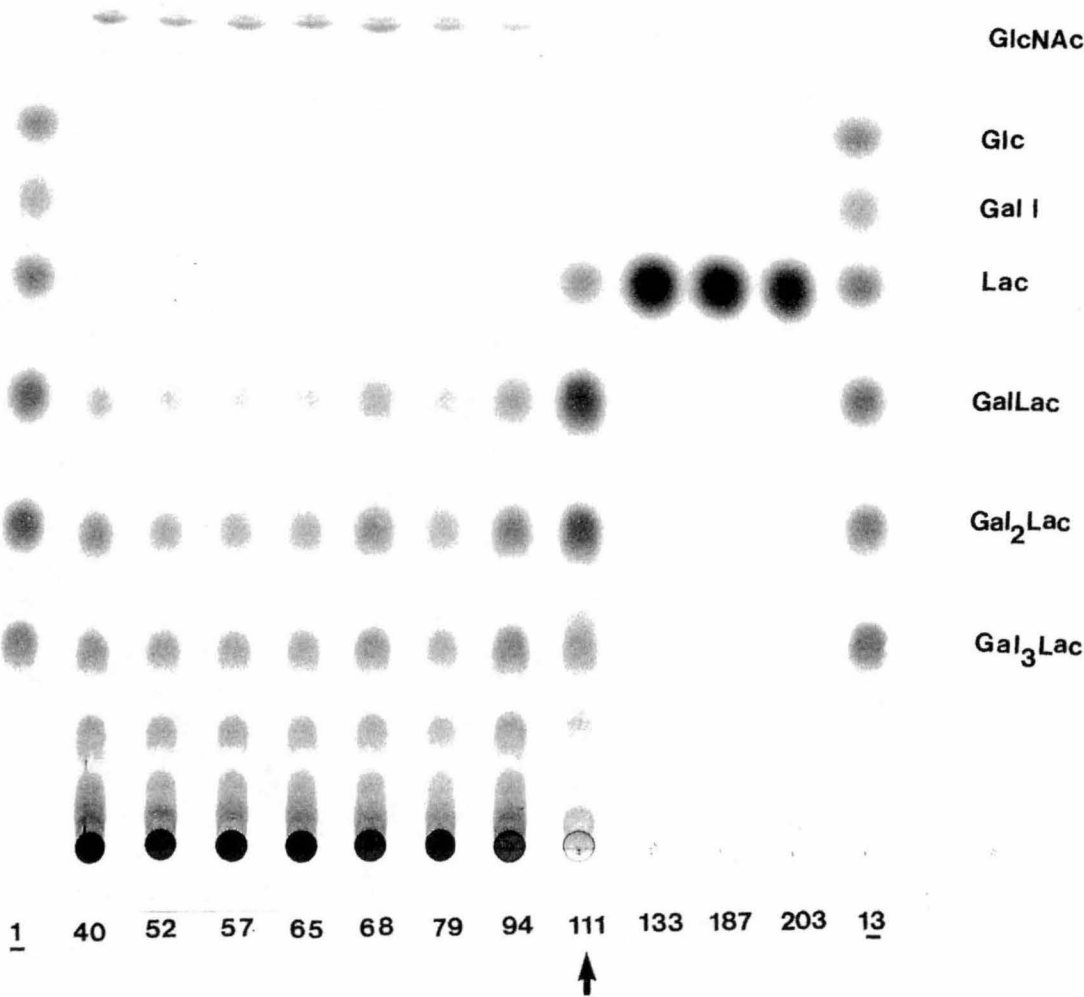
Figure 7.9

Thin-layer chromatogram showing carbohydrates in milk from the ringtail possum during Phases 2b and 3 of lactation.

1 and **13** are a mixture of standards containing:

- GlcNAc, N-acetylglucosamine;
- Glc, glucose;
- Gal, galactose;
- Lac, lactose;
- GalLac, 3'-galactosyllactose;
- Gal₂Lac, 3',3"-digalactosyllactose;
- Gal₃Lac, 3',3",3'''-trigalactosyllactose.

The numbers refer to days of lactation and pouch emergence by the young is indicated by the arrow.



containing: 3',3'',3'''-tri-galactosyllactose; 3',3''-di-galactosyllactose and 3'-galactosyllactose. The monosaccharides, glucose and N-acetyl glucosamine, are also present during this phase.

At 16 weeks post-partum, when some young are beginning to emerge from the pouch, lactose first appears in the milk. Lactose replaces the oligosaccharides by 19 weeks post-partum so that between 19 and 29 weeks post-partum or Phase 3 of lactation, when the young are out of the pouch, the milk contains only the disaccharide lactose and no monosaccharides or oligosaccharides.

Lipid

Values for milk lipid (g/100ml) were obtained from the standard curve determined from a plot of the Roese-Gottlieb extraction of lipid against the creamatocrit method (Fig. 7.2). The creamatocrit technique gave lipid values (%) which were higher than the values obtained by the gravimetric technique (g/100ml). This was also found by Hudson *et al.* (1979). There are two explanations for the higher creamatocrit values. Firstly, creamatocrit is a relative volume estimate whereas the Roese-Gottlieb technique is an accurate measure of mass and, since lipid has a specific gravity of less than one, a given mass of lipid will have a greater volume. Secondly, the cream layer probably includes materials other than lipids (e.g. water, some casein) which would also increase the creamatocrit volume.

Figure 7.7c shows the change in the lipid content of milk collected from the captive animals throughout lactation. Levels of lipid remain low throughout lactation when compared with the protein and total hexose levels. During early lactation from week 5 to week 10 lipid levels were about 1.5 g/100ml. After week 10 lipid levels rose steadily to an average peak of 3.8 g/100ml at week 16 of lactation before declining again following the emergence of young from the pouch. Levels of lipid remain low at 1.5 g/100ml between week 18 and week 23. Thereafter, levels increased with marked fluctuations towards the end of lactation.

The concentrations of lipid in individual milk samples collected from ringtails in the wild are also shown on Fig 7.7c. As with the other milk constituents, the milk lipid levels of wild milk samples appear to follow the same trends as in captive animals, during Phase 3 of lactation. However, lipid levels in wild milk samples collected during Phase 3 of lactation were found to be significantly higher than levels in captive Phase 3 milk (un-paired Student's t-test, Table 7.3).

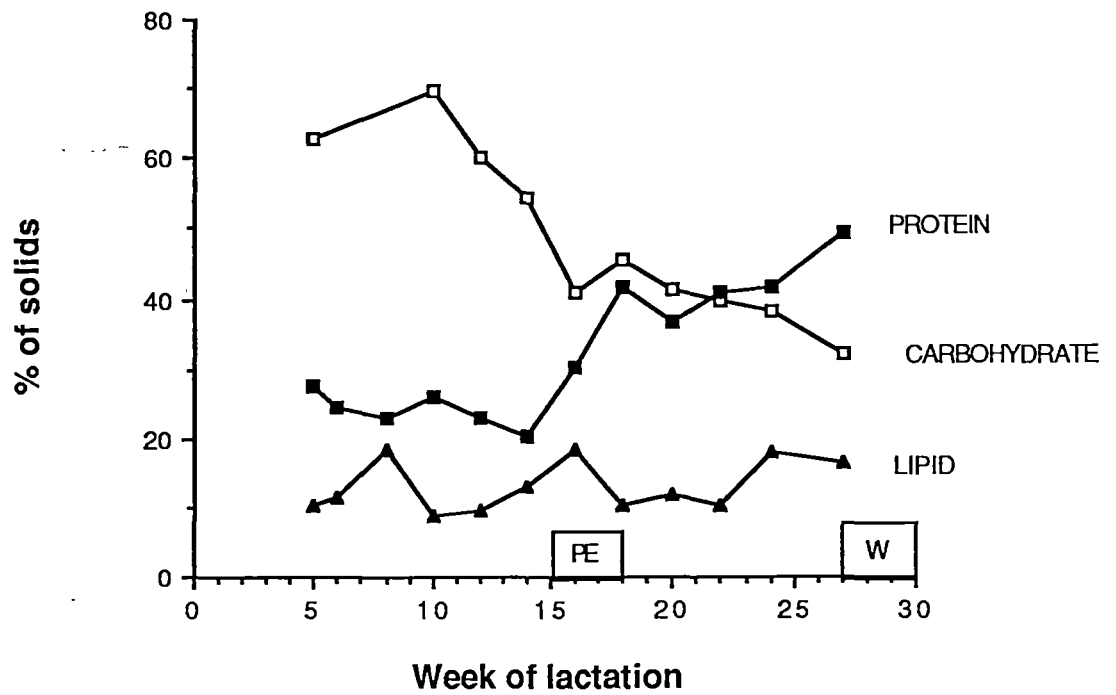
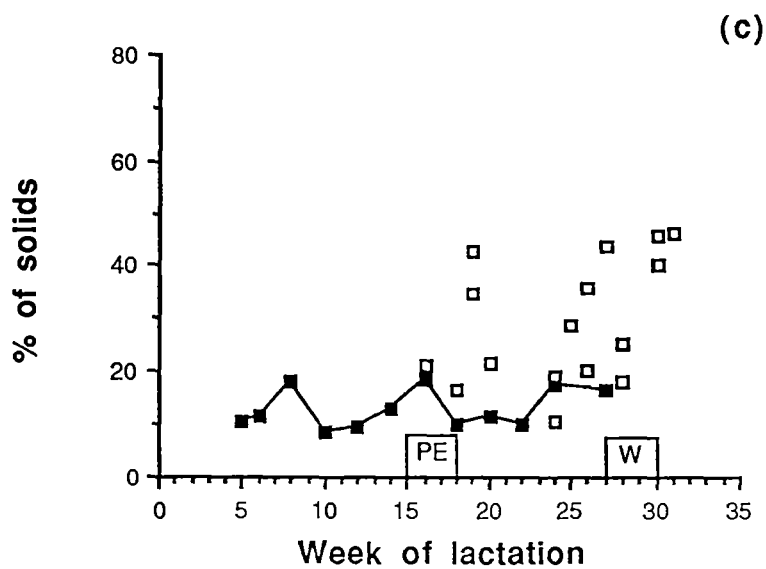
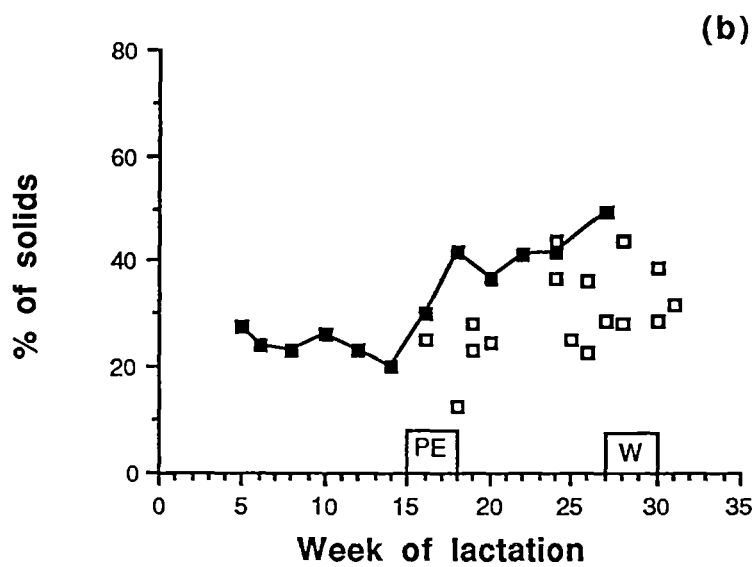
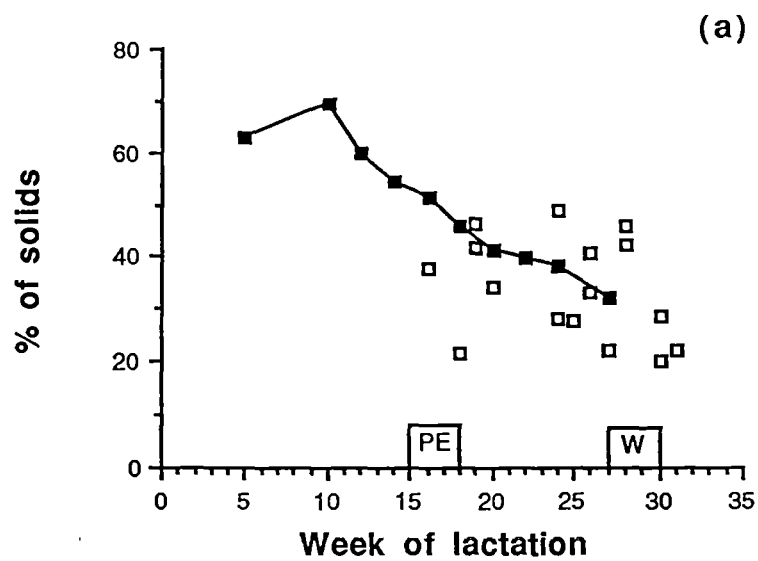


Figure 7.10 Relative proportion of carbohydrate, protein, and lipid throughout lactation expressed as a percentage of the solids fraction (w/w).

Figure 7.11 Relative proportion of **(a)** carbohydrate, **(b)** protein and **(c)** lipid (all expressed as a percentage of the solids fraction) in individual milk samples from wild *P.peregrinus* □ compared with values obtained for milk from captive *P.peregrinus* ■.



Carbohydrate: Lipid: Protein Ratios

The relative contributions of carbohydrate, lipid and protein to the solids fraction of the milk were calculated (Figure 7.10). From week 5 to week 10 of lactation total hexose constituted around 65% of the total solids fraction while protein and lipid were about 27% and 8% respectively. Between week 10 and week 22, total hexose begins to decline while the protein level slowly increased. At week 22 of lactation carbohydrate represented 39% of the solids fraction whereas protein had risen to 41%. From week 22 to the end of lactation protein continued to rise slowly while carbohydrate declined. The lipid contribution remained low throughout lactation rising only slightly to around 16% towards the end of lactation, at which stage protein represented approximately 49% of the solids fraction and, carbohydrate approximately 32%.

The relative contribution of carbohydrate, lipid and protein to the solids fraction of field milk was calculated for individual samples (Figure 7.11). When compared with the percentage contribution of carbohydrate to the solids fraction found in captive milk the percentage contribution of carbohydrate to the field milk solids follows the same trend dropping steadily from around 40 % to 20 % at the end of lactation. However the percentage contribution made by protein to the field milk solids fraction, increasing from around 20% to 35%, remains lower throughout phase 3 of lactation compared to captive milk protein. The lipid contribution to total solids is higher in field milk throughout phase 3 of lactation than in captive milk .

Electrolytes

Sodium concentrations were about 35 mM between week 5 and week 10 of lactation but dropped to about 15 mM, during pouch emergence and the initial phase of weaning (Figure 7.12a). Thereafter they began to increase gradually at week 21 to reach a peak of about 55 mMol at the end of weaning. This peak corresponded with final weaning (independence) of the young and regression of the mammary gland. Potassium levels showed no obvious change throughout lactation, fluctuating about the average level of 22mMol (Figure 7.12b).

Sodium levels in Phase 3 milk from field animals, although generally lower, were not significantly different to those found in milk from captive females at the same stage (Figure 7.12a, Table 7.3). However, the increase in sodium during the

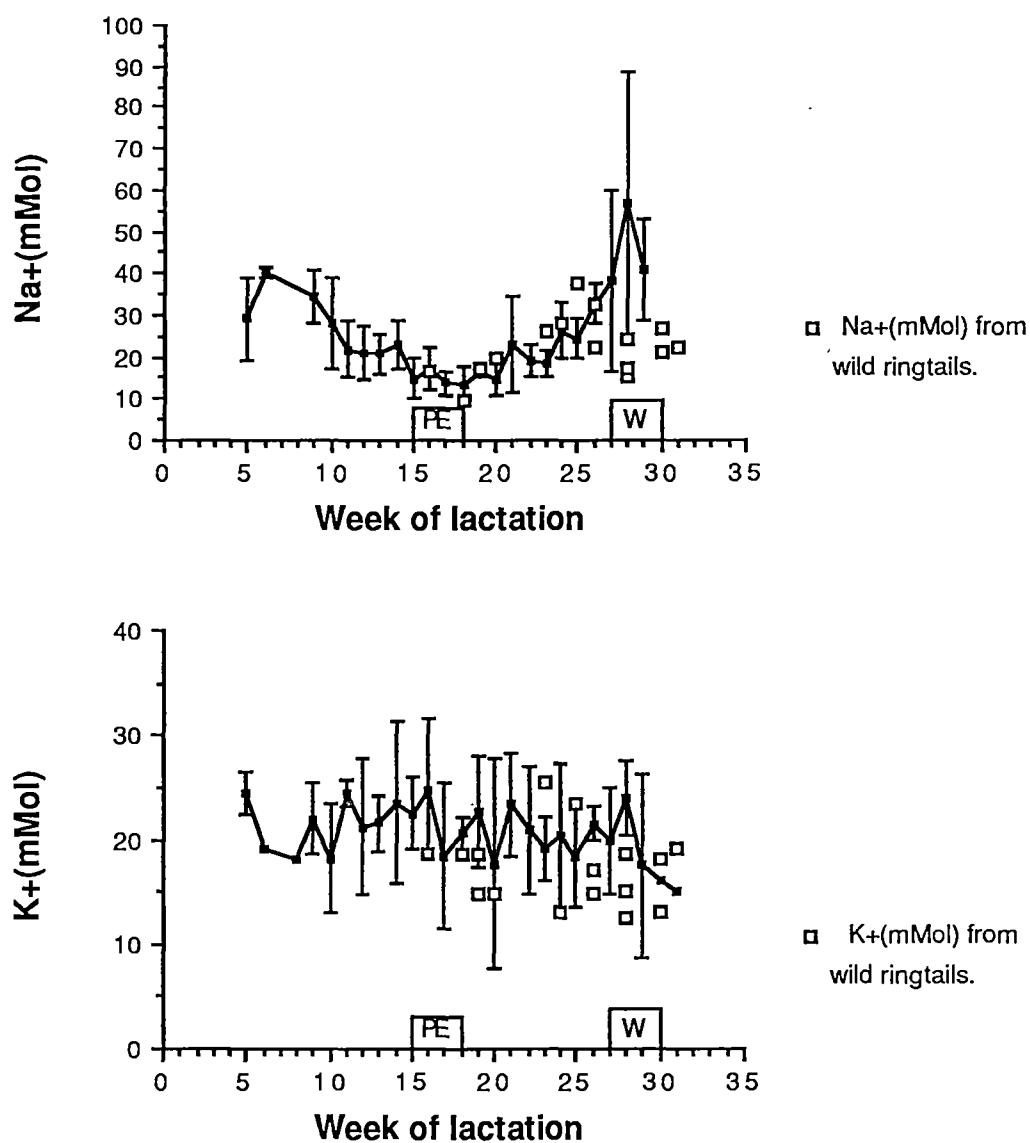
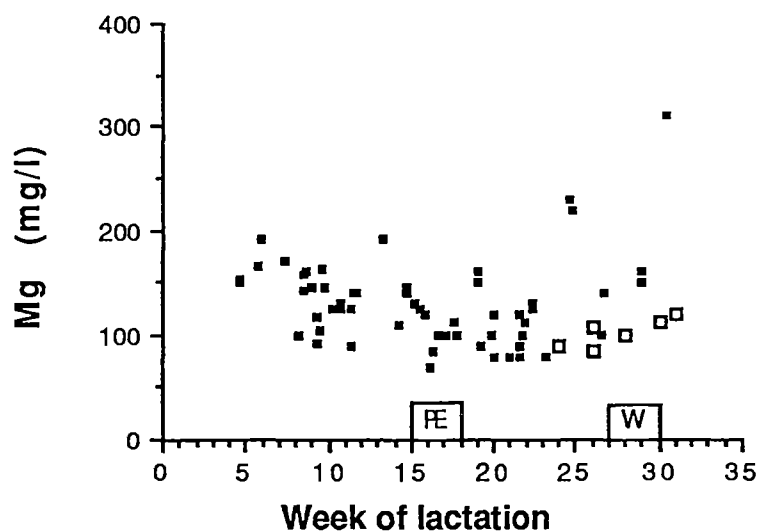


Figure 7.12 Levels of (a) sodium, $n = 15$, and (b) potassium, $n = 16$, in individual milk samples from wild ringtail possums (□), compared with values obtained for captive ringtail possums (■). (means \pm s.d., $n = 122$). PE = Pouch emergence, W = Final weaning.

(a)



(b)

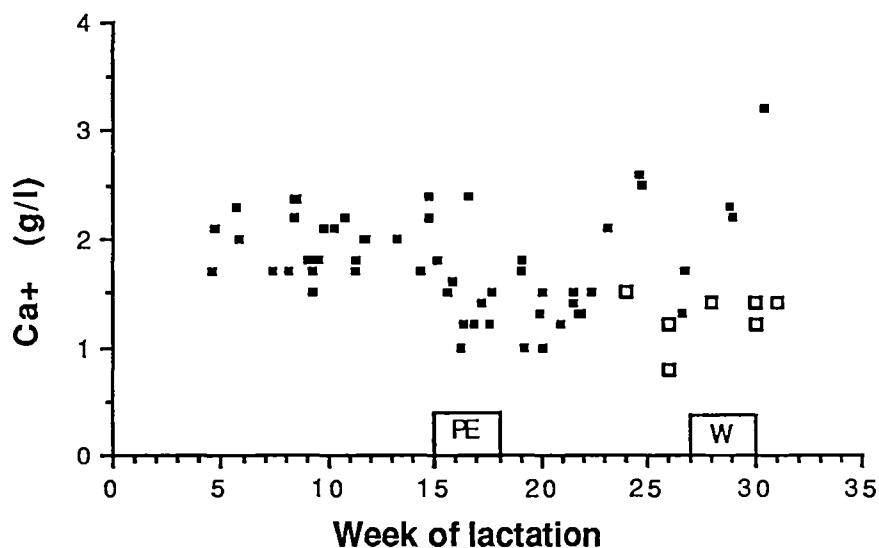


Figure 7.13 Levels of (a) magnesium, $n = 6$, and (b) calcium, $n = 7$, in individual milk samples from wild ringtail possums (\square) compared with individual values obtained for captive ringtail possums (\blacksquare) throughout lactation. PE = pouch emergence, W = Final weaning.

Table 7.4 Comparison of milk composition in paired mammary glands.

ANIMAL F1

Milk Constituent	Paired t-Test		
	df	t	P
carbohydrate	4	2.238	>0.05
protein	3	1.385	>0.05
solids	4	1.793	>0.05
fat	4	-0.040	>0.05

ANIMAL F2

Milk Constituent	Paired t-Test		
	df	t	P
carbohydrate	4	0.658	>0.05
protein	4	1.707	>0.05
solids	5	-0.892	>0.05
fat	4	0.756	>0.05

final weaning stage was not found in the field milk samples. The potassium concentration in milk collected from wild animals was found to be significantly lower than in milk collected from captive animals ($df = 102$, $t = 2.76$, $P < 0.05$; Figure 7.12b, Table 7.3).

Calcium and magnesium values were obtained for milk samples collected during the 1987/88 breeding season. Figure 7.13a and b shows individual values for milk magnesium and calcium throughout lactation. During early lactation calcium levels remained fairly constant around 2 g/l until the young started to emerge from the pouch when there was a slight decrease. Although the results are sparse towards the end of lactation, calcium levels appear to rise slightly to 3 g/l. Magnesium levels were generally lower than calcium levels but followed the same trend throughout lactation. Levels started at about 150 mg/l during early lactation, dropping slightly during pouch emergence and then increasing during late lactation reaching a maximum value of 300 mg/l at final weaning.

Calcium and magnesium were only determined for milk collected from field animals during the 1987/88 season. The concentrations of magnesium and calcium in milk from field animals appear to be similar to those of milk from captive females for the weeks of lactation covered (Figure 7.13, Table 7.3). However, the magnesium and calcium values obtained for the field samples during the final weaning stage do not show the slight increase found in milk from captive females.

Inter-Mammary Comparisons

The composition of milk sampled from right and left posterior mammary glands of female F1 and F4 was compared (Table 7.4). The results suggest that there is no significant difference between the composition of the milks produced in individual active mammary glands of a female.

7.4 Discussion

7.4.1 The Mammary gland

The increase in size of the ringtail possum mammary gland during Phase 2 of lactation is comparable to that which occurs in *Macropus eugenii* (Findlay, 1982a; Green, 1984), *Potorous tridactylus* (Crowley, 1984), and *Macropus agilis* (Lincoln and Renfree, 1981). The ultrastructure and histology of the ringtail

mammary gland is not known in detail, however, Stewart (1984) has shown that hyperplasia and hypertrophy of the alveoli cause the mammary gland size increase in *M. eugenii*. This increase in the alveolar tissue and the lumen size of the alveoli as the gland enlarges, has been noted for other marsupial species (*M. rufus*, Griffiths *et al.*, 1973; *M. agilis*, Lincoln and Renfree, 1981; *P. tridactylus*, Crowley 1984). There also appears to be a direct relationship between mammary size and milk yield (Hanwell and Peaker, 1977). Factors such as sucking and the associated release of lactogenic hormones and milk removal, are obviously important in maintaining and increasing production in the lactating gland (Findlay and Renfree, 1984). The increase in mammary gland size in the ringtail possum probably resulted from an increase in lumina size to enable high milk production and storage. The peak in size of the gland at the time when the young emerge from the pouch may reflect the high nutrient demands of the young at this stage of their development

The gradual decrease in size of the ringtail mammary gland after vacation of the pouch by the young occurs at an earlier stage to that seen in other marsupials. Intermittent sucking and milk removal by the young at this stage may lead to a reduction of milk synthesis by the gland. By final weaning the gland has regressed almost to an inactive state. It is during this final stage that a large reduction in secretory tissue occurs, possibly by cell disintegration and lysis (Pitelka and Hamomotu, 1983).

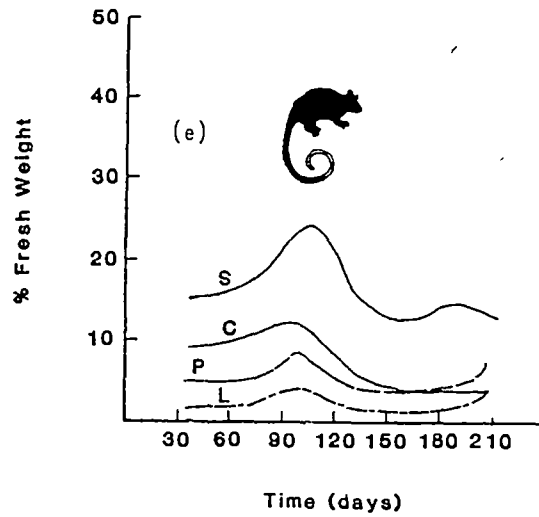
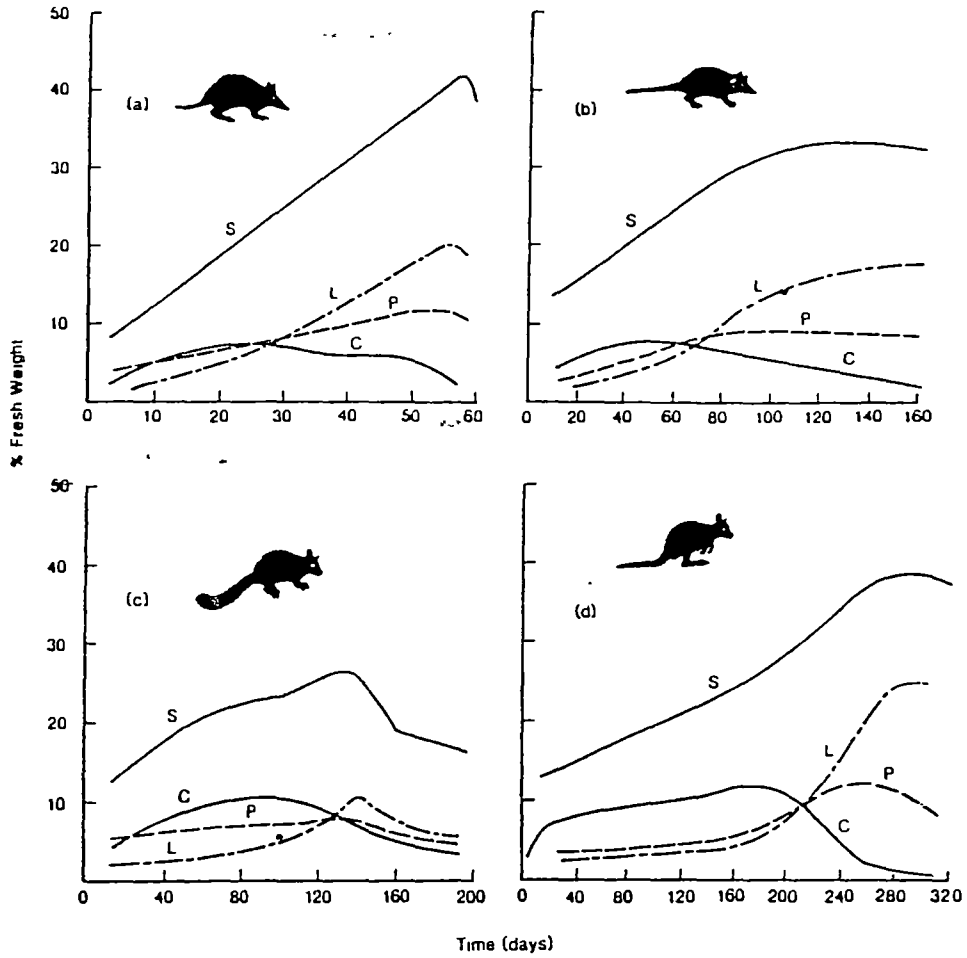
7.4.2 Milk Composition

The milk of the ringtail possum exhibits an increase in the concentration of solids during Phase 2b of lactation similar to the pattern shown by all marsupials studied to date (Green, 1984; Green *et al.*, 1987; Crowley *et al.*, 1988; Smolenski and Rose, 1988; Merchant and Libke, 1988; Merchant, 1989; Cowan, 1989). However, the drop in percent milk solids which occurs at the stage of lactation corresponding with pouch emergence, and stabilisation at a low level during Phase 3 of lactation has been observed in only three other marsupials, *M. rufa* (Lemon and Barker, 1967), *D. viverrinus* (Green *et al.*, 1987) and *T. vulpecula* (Cowan, 1989). The milk solids of *Isoodon macrourus* (Merchant and Libke, 1988) also decrease, but not until 2 to 4 days before lactation ceases (Fig. 7.14). The decline in milk solids in the ringtail possum occurred at an earlier stage in lactation than in

Figure 7.14: The proximate composition of milk (%w/w) in:

- (a) *Isodon macrourus* (omnivorous),
- (b) *Dasyurus viverrinus* (carnivorous),
- (c) *Macropus eugenii* (herbivorous),
- (d) *Trichosurus vulpecula* (folivorous),
- (e) *Pseudocheirus peregrinus* (folivorous),

S = solids, L = lipids, P = proteins, C = carbohydrates.
(Adapted from Green and Merchant, 1988; data for *P. peregrinus* from this study)



T. vulpecula (Cowan, 1989), although both corresponded to the time of pouch emergence which occurs slightly earlier in the ringtail possum. The concentration of solids in ringtail possum milk was generally lower throughout lactation than in other marsupials studied (Table 7.5, Figure 7.15), except for the red kangaroo, *M. rufus* (Lemon and Barker, 1967).

Ben Shaul (1962) and Jenness and Sloan (1970) noted that mammalian species which suckle frequently produce a dilute milk whereas those that suckle infrequently produce concentrated milk. In most marsupials the changes in milk solids and the size of the mammary gland during lactation are associated with changes in the sucking regime of the young. This is best illustrated in the tammar wallaby (Green *et al.*, 1980) where the milk concentration and gland size increases as sucking frequency decreases. Therefore, several authors have suggested that the pattern of milk composition within marsupial species appears to fit this hypothesis (Green *et al.*, 1980; Green, 1984; Cowan, 1989) and this has led to the idea that changes in marsupial glands and milk synthesis are correlated with sucking frequency. The changes seen in the ringtail mammary gland and milk solids up to pouch emergence also appear to fit the sucking hypothesis. The gland size and milk solids increase, after the young release the teat, when sucking becomes intermittent but probably of high intensity. However, unlike the tammar wallaby, when sucking is reduced to brief intermittent bouts, after the ringtail suckling has left the pouch, the gland decreases in size and milk concentration declines.

Tyndale-Biscoe and Renfree (1987) point out that although the growth of the marsupial gland may conform to the sucking theory, pouch-young transfer experiments (Findlay, 1982b) show that changes in the composition of the milk are an intrinsic characteristic of the mammary epithelial cells and that altered rates of sucking produce only transitory effects. The changes seen in ringtail milk solids are caused by the different rates of biosynthesis and secretion of the milk components (carbohydrate, protein, fat and electrolytes) during lactation. The patterns of change in the concentration of some of these components (i.e., carbohydrate, protein and electrolytes) throughout lactation were similar to those seen in the milks of other marsupials (Green, 1984; Green and Merchant, 1988). However, some differences are apparent between ringtail possum milk and other marsupial milks, particularly in the timing and magnitude of these changes.

Hexose levels in ringtail milk during early, middle and late lactation were

Table 7.5 Concentration of solids, hexose, protein and lipids in the milk of marsupials. (Diet: I-insectivorous, H-herbivorous, C-carnivorous, F-folivorous, M-mycophagous)

Species	Diet	% (w/w)												Reference
		Solids			Hexose			Protein			Lipid			
		E	M	L	E	M	L	E	M	L	E	M	L	
Tasmanian devil (<i>Sarcophilus harrisii</i>)	C	-	-	45	-	-	5	-	-	6	-	-	30	Green <i>et.al.</i> (1987)
Eastern native quoll (<i>Dasyurus viverrinus</i>)	C/I	15	30	34	5	7	2	3	9	8	4	15	15	Green <i>et.al</i> (1987)
Northern brown bandicoot (<i>Isododon macrourus</i>)	I	8	30	40	2	8	1	2	14	2	2	15	25	Merchant and Libke (1988)
Long nosed potoroo (<i>Potorous tridactylus</i>)	MI	17	35	50	9	15	2	5	12	15	2	10	26	Crowley (1988)
Tasmanian bettong (<i>Bettongia gaimardi</i>)	MI	25	25	40	10	12	2	8	11	16	3	6	20	Smolenski and Rose (1988)
Common wombat (<i>Vombatus ursinus</i>)	H	23	-	51	12	-	4	4	-	9	6	-	28	Green (1984)
Tammar wallaby (<i>Macropus eugenii</i>)	H	12	30	40	7	13	2	4	13	12	2	6	23	Green (1984)
Red kangaroo (<i>Macropus rufus</i>)	H	12	25	12	-	-	-	3	7	5	1	7	5	Lemon and Barker (1967)
Red-necked wallaby (<i>Macropus rufogriseus</i>)	H	16	25	27	8	11	2	4	7	10	3	7	14	Merchant <i>et al.</i> (1989)
Sugar glider (<i>Petaurus breviceps</i>)	I	-	-	31	-	-	11	-	-	8	-	-	22	Green (1984); S.Munks and A.Bradley (unpublished data).
Common brushtail possum (<i>Trichosurus vulpecula</i>)	F	10	28	18	5	11	5	4	8	5	3	9	5	Cowan (1989)
Koala (<i>Phascolarctos cinereus</i>)	F	-	-	35	-	-	5	-	-	6	-	-	18	Green (1984)
Common ringtail possum (<i>Pseudocheirus peregrinus</i>)	F	15	25	13	10	13	5	4	8	5	2	4	2	This study

E=Phase 2 milk; M=period covering pouch emergence; L=phase 3 milk. (adapted from Green, 1984)

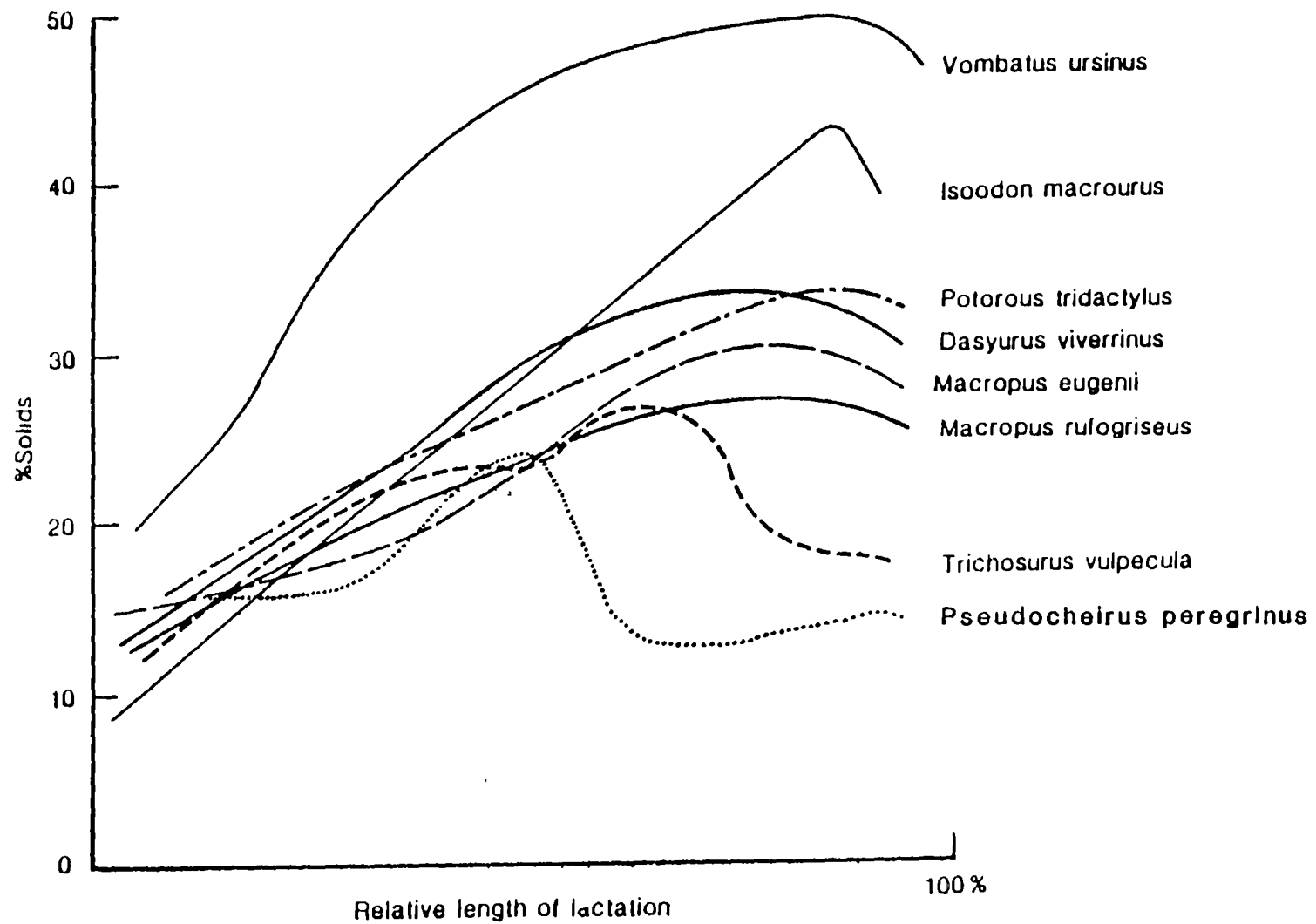


Figure 7.15

Percentage of milk solids (W/W) throughout lactation in marsupials (adapted from Green and Merchant, 1988)

intermediate in the range of marsupial values (Table 7.5). However, the precipitous drop of levels to about 1% during Phase 3 of lactation, seen in some marsupials such as *M.eugenii* (Green *et al.*, 1980) and *B.gaimardi* (Smolenski and Rose, 1988) is not apparent in the ringtail possum. Cowan (1989) has also noted a less pronounced decline in milk hexose in brushtail possum milk.

Qualitative analysis of ringtail possum milk carbohydrates showed that during Phase 2b of lactation the predominant carbohydrates in the milk consisted of higher oligosaccharides similar to those found for other marsupials (Green and Merchant, 1988). The precipitous decline in milk carbohydrate shown by most marsupial species studied, so far, is accompanied by a change from oligosaccharides to disaccharide and monosaccharide sugars. In the milk of *M.eugenni* (Messer and Green, 1979), *D.viverrinus* (Messer *et al.*, 1987) and *M. rufogriseus* (Green and Merchant, 1988) the monosaccharides galactose, glucose and glucosamine predominate toward the end of lactation. However, the decline in carbohydrate during pouch emergence by the ringtail young is accompanied by oligosaccharide sugars being replaced entirely with the disaccharide lactose. This situation has also been found in *T.vulpecula* milk (Cowan, 1989).

An important restriction on milk composition is the apparent necessity for the osmotic pressure of milk to be close to that of blood (Jenness and Sloan, 1970). The milk's osmolarity is determined mainly by its carbohydrate and salt content (Linzell and Peaker, 1971). A much higher level of hexose is achieved in marsupial Phase 2 milk, where oligosaccharides predominate, than in eutherian milk where the predominant carbohydrate is the disaccharide lactose. This is possible because oligosaccharides permit a much higher concentration of sugars within the constraints of osmotic equilibrium with body fluids, than could be achieved with equivalent concentrations of monosaccharides or disaccharides (Green and Merchant, 1988). Janssens and Ternouth (1970) suggest that the necessity for high levels of carbohydrate in marsupial milk, particularly herbivores during Phase 2 of lactation, are related to the ability of the pouch young to digest milk lipids

In the same way that oligosaccharides have a lower osmolarity than a similar amount of disaccharides, the disaccharide lactose has a lower osmolarity than a similar amount of monosaccharides. Therefore, for a given osmotic increment, nearly twice the concentration of lactose (compared with monosaccharides) may be accommodated and hence twice the calorific value (Jenness and Sloan, 1970). The

relatively higher level of carbohydrate found during Phase 3 of lactation in the ringtail possum and *T.vulpecula* milk compared with other marsupial milk may, therefore, be related to the presence of the disaccharide, lactose instead of monosaccharides. The low levels of fat in the ringtail possum milk at this stage suggests that carbohydrate may be needed in higher concentrations at this stage, to maintain the calorific value of the milk. The absence of oligosaccharides during Phase 3 of lactation to achieve the same end is probably due to the change in the activity of the intestinal digestive enzymes. Janssens and Messer (1988) suggest that the changes in the activities of these enzymes are related to the dietary transition from milk sugars to complex carbohydrates in the solid diet during Phase 3 of lactation.

The absence of significant amounts of lactose in macropod milk and the observation that when young kangaroos are bottle-fed with milk substitutes containing lactose they suffer severe diarrhoea, led to an investigation of lactose absorption in macropods (Crisp *et al.* 1987). They found that lactose in macropods is hydrolysed intracellularly by an acid beta-galactosidase, probably located in the lysosomes, instead of by a neutral beta-galactosidase attached to the external surface of the brush border membrane of the intestine, which system operates in eutherians. Transport of lactose into the macropod enterocyte is probably much slower than hydrolysis on the external surface of the intestinal lumen. Therefore a build up of lactose in the intestinal lumen when macropods are fed a high lactose milk exerts an osmotic effect sufficient to produce diarrhoea (Crisp *et al.*, 1987).

A recent study on the mechanism of lactose absorption in *Trichosurus vulpecula* has shown that an extracellular neutral beta-galactosidase is present on the intestinal brush border of this species (Crisp *et al.*, 1989). The major enzyme responsible for hydrolysing the milk sugars during Phase 2 of lactation in the brushtail is the acid beta-galactosidase, similar to that found in macropodid milk (Crisp *et al.*, 1987). However, the major enzyme responsible for hydrolysing milk sugars during Phase 3 of lactation was shown to be the neutral beta-galactosidase (Crisp *et al.*, 1989). This, in conjunction with the possibility that intestinal pinocytosis ceases during the weaning period in *T.vulpecula* as in many suckling eutherians, has lead to the suggestion that lactose is hydrolysed extracellularly on the intestinal brush border by neutral beta-galactosidase (Crisp *et al.*, 1989). This proposed mechanism for the absorption of lactose during Phase 3 of lactation in

T. vulpecula suggests that this species would not exhibit the effects of lactose intolerance seen in suckling macropodids. The observation that the carbohydrate of ringtail possum Phase 3 milk is made up entirely of lactose, as in brushtail milk, makes their mechanism of lactose absorption an important area for further investigation.

No clear explanation for the differences between milk protein levels found for 1986/87 females and 1987/88 females is available; they may be due to storage in the refrigerator, milking process and/or individual variation. Although stored at -20°C protein breakdown in the milk can still occur, depending on the storage time, and 1986/87 samples were stored for slightly longer than 1987/88 samples. However, lipid is usually most affected by increased storage time (Woodward, pers comm.) and lipid levels did not differ between the two years. Vigorous milking can cause tissue fluid to move into milk ducts (Ling *et al.*, 1961), however, carbohydrate, sodium and potassium levels would also be expected to vary if this was the case. Variation of milk composition between individuals may relate to the diet and general condition of the animal, e.g. Forsum and Lonnerdal (1980) showed that in humans dietary protein levels affected milk protein content. Ringtail possums maintained in captivity during 1987/88 could have received more of the supplementary protein rich mixture (see Chapter 2) than animals during 1986/87. Thus, some or all of these factors may be responsible for the differences in protein levels for the two years, despite the fact that efforts were made to maintain standard milking techniques and analytical procedures.

Levels of protein in ringtail possum milk at early, middle and late lactation were similar to those of other marsupials e.g., *M. rufus*, *V. ursinus*, *D. viverrinus* and *T. vulpecula* (Table 7.5). The increase in protein concentration during the final weaning stage of lactation seen for both years is likely to be a result of mammary involution. As mentioned earlier, regression of the mammary gland at the cessation of lactation involves cell lysis and disintegration (Pitelka and Hamamotu, 1983) and therefore some cellular material could be discharged along with the milk, resulting in elevated protein levels.

Casein and whey proteins are present in marsupial milk with whey representing the predominant milk protein fraction (Green and Renfree, 1982). The protein content measured in this study was a combination of the casein

and whey fractions. An investigation on *M. eugenii* showed 50-85% of milk proteins to be in the whey and that these proteins varied qualitatively with time (Green and Renfree, 1982) whereas the proteins in the casein fraction showed no qualitative change. The electrophoretic pattern of whey protein has been found to change throughout lactation in a number of other marsupial species, e.g. *S. brachyurus*, Jordan and Morgan (1968); *M. rufus*, Lemon and Bailey (1966); *M. giganteus*, Lemon and Poole (1969); *M. eugenii*, Green and Renfree (1982) and *P. tridactylus*, Crowley (1984). The number and intensity of protein bands increases as lactation proceeds, particularly in the alpha-globulin and pre-albumin band (Green and Renfree, 1982).

Renfree *et al.* (1981) have shown that while the relative concentration of some amino acids are constant during lactation others predominate at different stages and are presumed to be linked to the developmental requirements of the young (e.g. higher levels of sulphur-rich amino acids during hair growth). One of the most interesting changes in the milk proteins is the appearance of specific whey proteins during Phase 3 of lactation. The appearance of these late stage proteins has been observed electrophoretically in *S. brachyurus* (Jordan and Morgan, 1968) and *M. eugenii* (Green and Renfree, 1982) and two were isolated from the milk of *M. rufus* and *M. giganteus* (McKenzie *et al.*, 1983). McKenzie *et al.* (1983) suggest that one of these milk proteins, found in *M. giganteus* was an alpha-lactalbumin and the other a beta-lactoglobulin. However, Nicholas *et al.* (1987) isolated and partially characterised one late stage protein from the milk of *M. eugenii*. This new protein was named LLP-A and Nicholas *et al.* (1987), suggested it was homologous with the protein identified by McKenzie *et al.* (1983) as an alpha-lactalbumin in *M. giganteus* milk. The appearance of LLP-A during Phase 3 of lactation in *M. eugenii* coincides with the young leaving the pouch. This led Nicholas *et al.* (1987) to suggest that this protein may have a role in gut development as the young changes from an exclusive diet of milk to an increasingly herbivorous diet.

Electrophoresis of ringtail whey proteins revealed the presence of two low molecular weight proteins during Phase 3 of lactation, a lysozyme and alpha-lactalbumin. Alpha-lactalbumin was present in all samples of milk between 98 and 187 days of lactation, which covers the period of transition from Phase 2 milk to Phase 3 milk. Alpha-lactalbumin forms part of the enzyme involved in lactose

synthesis (Groves, 1971). This protein is found in macropod milk throughout lactation but only in small amounts during late lactation when macropod milk oligosaccharides decline. The relatively larger amounts of alpha-lactalbumin in ringtail milk during Phase 3 of lactation are probably related to the presence of lactose. It is well established that alpha-lactalbumin and lysozyme are structurally related but exhibit a clearly defined functional divergence (Hill and Brew, 1975). Lysozyme has been shown to be present in reptiles, birds and eutherian mammals (Stewart *et al.*, 1987) and it has been postulated that it is the ancestral gene for alpha-lactalbumin. Lysozyme has only been found in one other marsupial, *M. giganteus* (McKenzie *et al.*, 1983).

The appearance of lysozyme in *P. peregrinus* milk between 112 and 140 days of lactation correlates with pouch emergence at the time when the young begins to eat foliage. Its time of appearance resembles that of LLP-A in mid-lactation in *M. eugenii* milk (Nicholas *et al.*, 1987). Lysozyme is an enzyme involved in the breakdown of b-1,4 glycosidic linkages in the polysaccharides of bacterial cell walls, therefore it may have a role in preventing bacterial infection in the suckling. This was not found in baboons, *Papio cynocephalus* but in these animals it has been suggested that lysozyme in the milk may have some influence on the young's intestinal flora or have a role in preventing infection in the lactating mammary gland (Buss, 1969). The lysozyme in ringtail possum milk may also have a role in hydrolysing the bacteria ingested with the faeces during caecotrophy (Chapter 1), in the stomach, to provide an additional protein source for processing in the caecum. However, the time at which ringtail young first engage in caecotrophy is not known although the earliest observation made in this study was at 26 weeks of lactation. Alternatively the lysozyme may hydrolyse the bacteria in maternal faeces ingested by the young prior to the onset of caecotrophy. This ingestion of maternal faeces is generally thought to inoculate the young with protozoans essential for the digestion of foliage and has been observed in *Phascolarctos. cinereus* during a period of a few days before they start eating foliage (Fleay, 1937). Similarly, Pahl (1987b) noted finely chopped leaf particles in the faeces and stomach contents of ringtail possum pouch young, and suggested that these came from maternal faeces.

Crude lipid levels remained low throughout lactation in ringtail possum milk compared with other marsupial species. (Table 7.5 and Figure 7.15). A slight

increase to a peak at 15 weeks, which corresponded with the stage when some young were emerging from the pouch, was followed by a rapid decline to low levels. This pattern was different to that seen in other marsupials (Green, 1984; Green *et al.*, 1987; Smolenski and Rose, 1988; Crowley *et al.*, 1988; Cowan, 1989; Merchant, 1989) where lipid levels continue increasing as carbohydrate levels decline. The contribution of lipid to the solids fraction in ringtail milk was low throughout lactation and a lipid-carbohydrate crossover correlating with pouch vacating, which is seen in other marsupial species, was not apparent. However, the drop to low levels of milk lipid during Phase 3 of lactation in the ringtail resembles the drop seen in *T. vulpecula* during the last 30 to 40 days of lactation (Cowan, 1989). The higher lipid level in weaning milk, which has a characteristic thick, sticky consistency, is probably a result of the regressing mammary gland which resorbs water soluble compounds leaving fat to accumulate in the milk (Hartmann and Kulski, 1978).

The change in sodium levels throughout lactation in *the ringtail possum* was similar to that seen in other marsupials e.g. *S. brachyurus* (Bentley and Shield, 1962), *M. eugenii* (Green *et al.*, 1980), *D. viverrinus* (Green *et al.*, 1987), *P. tridactylus*, (Crowley *et al.*, 1989), *I. macrourus* (Merchant and Libke, 1989), *T. vulpecula* (Cowan, 1989). In other marsupials potassium and sodium concentrations tend to show an inverse relationship. However, this relationship was not found in ringtail possum milk. Nevertheless, the relative concentrations of sodium and potassium did change throughout lactation. Sodium concentration was higher than potassium in Phase 2 milk but at pouch emergence when sodium levels are at their lowest the relative concentration of potassium was higher.

Overall concentrations of milk sodium and potassium were lower in the ringtail possum than in any other marsupial studied so far. Green (1984) noted an inverse relationship between milk electrolytes and milk carbohydrates in marsupials, which keeps the milk isosmotic with plasma. Messer *et al.* (1987) observed that oligosaccharides of *D. viverrinus* are of a larger molecular size than those of *M. eugenii*. Therefore they exert lower osmotic pressures allowing higher levels of electrolytes in the milk (Green *et al.*, 1987). This relationship between the electrolyte concentration and the molecular size of the milk oligosaccharides is also apparent in ringtail possum milk, where during early lactation the concentration of small oligosaccharides increases as sodium levels drop.

7.4.3 Milk Composition of Free-living Ringtail Possums.

Few studies have been made comparing milk samples from a species in the wild and the same species maintained in captivity. Green *et al.*, (1980) found milk samples from wild *Macropus eugenii* to be similar both in absolute values and the trends they show throughout lactation. However, Jenness and Sloan (1970) noted lower levels of carbohydrate (lactose) in milk from bears in the wild when compared with milk from bears in zoos.

The changing levels of total milk solids in samples from wild ringtail possums during Phase 3 of lactation were similar to those found in milk samples from captive ringtails. Levels of milk carbohydrate and the percentage contribution made by carbohydrate to the milk solids fraction in wild and captive ringtail possums were also similar. However, despite the small sample size, the overall level of protein in milk samples from wild animals during Phase 3 of lactation appeared to be significantly lower than that of captive animals. Lipid levels also differed between wild and captive ringtail possum milk during Phase 3 of lactation. These higher levels of lipid corresponded with the lower levels of protein.

The mammary gland may use preformed amino acids, fatty acids and monosaccharides as well as synthesising these components within the gland for incorporation in the milk (Jenness and Sloan, 1970). Therefore, differences in the protein content of the diet may alter the synthesis of specific milk proteins which may in turn alter the total protein content of the milk (Hambraeus, 1984). Ringtail possums in captivity were fed *E. amygdalina* foliage and occasionally a supplementary high protein mixture (Chapter 2), whereas ringtail possums in the wild are feeding predominantly on *L. laevigatum* (Chapter 4). Therefore, the higher protein content of the captive diet may have resulted in a higher protein level in the milk. Qualitative analysis of field samples may reveal whether the difference in overall protein levels compared with captive milk is reflected in the whey proteins.

Similarly, food lipid can contribute to variation in total milk fats. Fatty acids of milk fats representing blends of dietary acids and those synthesised by the gland activity (Glass *et al.*, 1969). The influence of dietary fat on the composition of fat and total fat content of the milk has been shown in humans (Prentice, 1980). Work on a monotreme, the echidna *T. aculeatus* (Griffiths *et al.*, 1973; Griffiths, 1978;

Griffiths *et al.*, 1984) showed that the fatty acid composition of milk fat can be changed by offering food with different fatty acid compositions. Grigor (1980) suggested that marked differences in levels of linoleic acids found in the milk fat of a marsupial *T. vulpecula* could be due to the differences in the dietary status of the mother. Similarly, in a study on the milk of the numbat, *Myrmecobius fasciatus* the low level of oleic acid in the milk fat of captive animals compared with wild animals was attributed to differences in the fatty acid composition of the diet (Griffiths *et al.*, 1988). The composition of the fatty acids in ringtail possum milk is unknown but there may be more available metabolisable fat in the field diet (*L. laevigatum*) when compared with the predominantly *E. amygdalina* captive diet (see Chapter 4 and 5) which may have caused differences in the relative fatty acid composition of the milk and in turn, the total lipid content of the milk.

However, low milk fat, and some compensatory rise in protein, in domestic ruminants has been related to diets deficient in fibre (Van Soest, 1982). The mechanism behind this phenomenon is unclear, however, it appears that milk fat depression is involved with the regulation of fat metabolism i.e., low fibre diets promote deposition of fat rather than fat mobilisation and subsequent secretion in milk (Van Soest, 1982). The higher fat content of milk from the free-living ringtail possums may, therefore, be associated with the higher total cell wall (fibre) content of *L. laevigatum* foliage compared with that of *E. amygdalina* (see Chapter 4 and 5).

7.4.4 Factors Influencing Ringtail Possum Milk Composition Throughout Lactation

The major function of milk in all mammals is to provide nutrition for the growing young (Eisenberg, 1981). Three major factors influencing the milk composition and lactational strategy, which have evolved to perform these functions in a particular species, are

- (i) the growth and metabolic requirements of the young;
 - (ii) the physiological constraints on the mother; and
 - (iii) the restrictions imposed by the environment (i.e., diet, habitat).
- Jenness and Sloan (1970) suggested that the milk composition that has evolved in a particular mammalian species is the resultant of a combination of these factors. In marsupial species, with their long period of lactation, the relative importance of these factors

in influencing lactation may vary throughout lactation.

During pouch life the milk composition appears to primarily suit the physiological and anatomical constraints of the young. For example, the high carbohydrate levels during Phase 2 of lactation appear to meet the digestive capabilities of the young. Milk sugars, being more easily digested than fatty acids, are therefore the preferred energy source during pouch life. The peak of carbohydrate levels at pouch emergence are important for the increased activity of the pouch young at pouch emergence and physiological changes such as maintaining body temperature. Physiological constraints also lead to the importance of maintaining the isotonicity of milk and plasma. This enables the young to obtain an adequate water intake without suffering a water imbalance in early pouch life when it cannot concentrate its urine. The oligosaccharide nature of the carbohydrate enables high carbohydrate content without making the milk hyperosmotic.

The specific growth requirements of the young, such as hair formation, may be reflected in the protein content of the milk at this time. Bunge (1898, cited in Bjornhag *et al.*, 1979) proposed that growth rate and protein concentration of milk were closely interrelated. Several authors have looked at this hypothesis (Brody, 1945; Bernhart, 1961; Blaxter, 1961; Jenness and Sloan, 1970) but have been unable to prove its general validity. Bjornhag *et al.* (1979) suggests that the problem was in finding adequate measures for growth rate and nutrient supply for particular species so that comparisons can be made between them. They compared the relative growth rate (corrected for the influence of size on growth rate) and the milk composition expressed as mg protein/Kcal energy in fat and lactose and found a significant correlation in 30 mammalian species. The significant increase in the percentage contribution of protein to the solids fraction of ringtail milk at the time when the young are emerging from the pouch may, therefore, be related to the increased growth rate of the young at this stage (Chapter 3). However, although Bjornhag *et al.* (1979) have shown that interspecific trends correlate to growth rate, the relationship between protein and growth rates across lactation stages within a species needs to be validated.

At pouch vaciation the composition of ringtail possum milk and other marsupial milk shows marked qualitative and quantitative changes and there is a corresponding peak in size and activity of the mammary gland. The digestive system of the ringtail possum suckling is changing dramatically at this time. In herbivorous marsupials studied to date (Griffiths and Barton, 1966; Janssens, 1984; Hume, 1982) this change is from digestion using endogenous enzymes to microbial fermentation at the time when herbage becomes a significant part of the

diet (Janssens and Ternouth, 1970). A change in digestive physiology appears to be a major factor affecting the carbohydrate fraction of the milk (Janssens and Ternouth, 1977).

Increased energy requirements for thermoregulation and activity by the young, at the onset of pouch emergence, influence all the milk constituents. Even the contribution of fat to total solids, which is generally low throughout lactation, shows a small peak at this time. After the young have emerged from the pouch digestive changes may continue but behavioural changes are also probably taking place as the young ringtail possum adapts to its foliage diet and arboreal lifestyle. Milk is an important dietary supplement, at this stage, until the suckling becomes efficient at foraging and digesting solid food. Phenolic compounds are found in the milk of another folivore, *T. vulpecula*, and they increase in concentration from the time the young leave the pouch to weaning. This is believed to condition the young to the phenolic compounds it will have to cope with when eating *Eucalyptus* leaves (Janssens and Ternouth, 1987). Ringtail possum milk may similarly prepare the young for its leaf diet, when they have to tolerate and detoxify the phenolic compounds (see Chapter 4).

The intake of solid food during Phase 3 of lactation provides the ringtail young with an additional source of nutrients. Therefore the necessity for the milk to suit the nutritive and physiological requirements of the young may be relaxed. The Phase 3 milk perhaps simply provides a supplementary nutrient and energy source to the young as it adapts to its leaf diet, its composition reflecting the physiological constraints on the mother and environmental factors. For example, the low total milk solids content at this stage is mainly because of low milk fat which requires a large amount of maternal energy for synthesis (Stryer, 1975). The metabolic constraints on the ringtail mother due to the low nutritive value of her folivorous diet may have resulted in the selection for milk with a relatively low fat content.

In summary, the composition of ringtail possum milk shows some similarities and some differences to the milks of other marsupial species studied to date. In particular, ringtail possum milk is relatively dilute with a low concentration of fat. The diet of the female is suggested as one factor which may have influenced the selection of milk with such a composition. Furthermore, this study indicates that dietary factors may effect the concentration of milk protein and milk fat within a species.

This study reinforces the view that lactation in the various marsupial species has a common pattern but that this pattern can vary according to the lifestyle of a particular species (Merchant and Libke, 1988). However, before the lactational strategies of species with different lifestyles can be compared, knowledge of the actual amount of milk produced is needed (Jenness and Sloan, 1970). This would give more information on the energy cost of lactation to the female and allows the percentage intake of various milk components to be related to the growth rate of the young.

CHAPTER 8**MILK YIELD, MILK ENERGY OUTPUT AND ENERGETICS OF
GROWTH IN THE COMMON RINGTAIL POSSUM.****8.1 Introduction**

A major function of mammalian milk is to provide the young with adequate nutrition to enable growth (Eisenberg, 1981) while maintaining body homeostasis (Bentley and Shield, 1962). The energy content of milk must be sufficient to fuel the metabolic requirements of the developing young and provide nutrients that are needed as 'building blocks' for growth. Information on milk intake by the young, in conjunction with knowledge of the composition of the milk consumed, is required to elucidate factors influencing the pattern and rate of growth in any mammalian species (Holleman *et al.*, 1975; Green *et al.*, 1988). For example, the growth of young may be an indication of milk energy and nutrients, a reflection of the volume of milk consumed, or both. Green *et al.*, (1988) note that before the lactational strategies of placentals and marsupials can be compared effectively, studies on milk consumption/production rates and hence growth energetics of a range of marsupials need to be made.

The milk energy intake of the young is an index of the energy that mothers invest in their young (Ortiz *et al.*, 1984). A lactating female requires energy to synthesise milk for her young in addition to the energy required to satisfy her own maintenance needs. Environmental factors and/or maternal diet, may affect the energy available for lactation and hence indirectly influence milk production. For example, the low available energy content of leaves may lead to a low rate of milk yield by lactating folivores (Gittleman and Oftedal, 1987). However, effective comparison of the relationship between the lactational strategy and ecological niche requires knowledge of both milk production and composition in a range of mammalian species (Jenness and Sloan, 1970).

Milk consumption rates and growth energetics have been studied in many eutherian species (Barnicoat *et al.*, 1949; McCance, 1959; Carl and Robbins 1988; Wright *et al.*, 1974; Dove and Freer, 1979). In contrast, milk yield and growth energetics has only been examined, in detail, in one marsupial species, the tammar

wallaby, *Macropus eugenii* (Green, 1984; Green *et al.*, 1988; Cork and Dove, 1989; Dove and Cork, 1989).

Studies of milk production in eutherians have used a variety of techniques (reviewed by Linzell, 1972, Wright, 1982 and Oftedal, 1984). Early attempts generally involved gravimetric methods requiring removal of the young from the mother and subsequent weighing of the litter, mother or both after a period of suckling (Coombe *et al.*, 1960). These techniques are not particularly accurate in that they involve the extrapolation to free-living animals of an estimate obtained during a period of disturbance (Dove and Freer, 1979). Furthermore, in some mammalian species the mother may lick the anal and urethral region of the suckling and consume the excreta, which may result in underestimates of milk intake when gravimetric techniques are used (Green and Newgrain, 1979).

Other methods used to estimate milk production include calorimetry and measurement of the increase in mammary tissue mass or volume (Linzell, 1972). Macfarlane *et al.*, (1969) measured milk intake from the turnover of tritiated water in sucklings, when the young were taking milk as their only source of water. This technique has been used successfully in wild species, e.g.; deer (McEwan and Whitehead, 1971) and baboons (Buss and Voss, 1971). Linzell (1972) suggests that this technique is particularly useful; having a wide application and reducing the problems/limitations of the previous techniques. However, there are also potential errors associated with the use of the tritium isotope dilution technique to estimate milk intake (reviewed by Oftedal, 1984, 1985; Kunz and Nagy, 1988). Sources of such errors include:

- 1) incorporation of isotopes into non-exchangeable hydrogen sites in newly synthesised tissue,
- 2) reduction of isotope concentration due to increasing size of the body water pool (Dove and Freer, 1979),
- 3) recycling of isotopes from suckling to mother via urine and faeces of the young and the pulmocutaneous exchange of water between litter mates (Baverstock and Green, 1975; Friedman and Bruno, 1976).
- 4) entry of water into the body water pool from sources other than milk (i.e., intake of solids and/or metabolised fat).

An alternative isotopic technique using ^{22}Na (Green and Newgrain, 1979) enables estimates of sodium influx and hence milk intake to be made by measuring

the turnover of radioactive sodium in the suckling and the sodium content of the milk. The use of ^{22}Na avoids the problems of pulmocutaneous exchange associated with tritiated water (Green and Newgrain, 1979; Baverstock and Elhay, 1981). Furthermore, although the mother will consume ^{22}Na from injected young, less ^{22}Na is returned to the young via the milk than tritiated water, since sodium is in low concentration in the milk compared with extracellular fluids and represents only a small proportion of the mothers sodium pool (Green and Newgrain, 1979). Therefore, recycling of ^{22}Na via the milk insignificantly influences estimates of rate of milk production (Green and Newgrain, 1979; Baverstock and Elhay, 1981).

After pouch leaving the pouch the young obtains sodium from its solid diet as well as from milk. If the amount of sodium intake via the diet is unknown then the sodium technique ('single-isotope') cannot accurately measure milk intake (Green *et al.*, 1988). However, a 'double-isotope' method described by Holleman *et al.*, (1975) can be used to estimate milk intake at this stage of lactation. This technique simultaneously measures the total water turnover of the mother and the offspring using tritium and deuterium isotopes, respectively. The contribution of milk to the water turnover of the offspring is derived from the transfer of tritiated water from the mother to the suckling which solves the problem of the young getting water from elsewhere (Dove and Freer, 1979). The 'double isotope' technique (Holleman *et al.*, 1975) still potentially involves errors 1,2 and 3 noted in the use of the tritium isotope technique. Nevertheless, the first source of error in a slow growing animal (such as the ringtail possum, see Chapter 3) would be small. The second source of error may be eliminated by the use of appropriate equations (Dove and Freer, 1979). Furthermore, Dove *et al.*, (1989) have shown that errors associated with 'recycling' (error three) are small when the offspring's pool size and milk water inflow are a small proportion of the mother's pool size.

The 'double-isotope' method (Holleman *et al.*, 1975) has been used successfully with wild ruminants (*Odocoileus hemionus* and *Oreamnos americanus*, Carl and Robbins, 1987) lambs (Wright and Wolff, 1976; Dove, 1988) and the tammar wallaby *Macropus eugenii* (Cork and Dove, 1986,1989; Dove and Cork, 1989). It is particularly useful in the measurement of milk intake during a prolonged period of lactation in timid species where extensive handling is not advisable.

The previous chapter examined the composition of ringtail milk throughout

lactation. The aim of this chapter was to obtain estimates of milk intake in the ringtail possum during pouch life and after pouch emergence, using the ^{22}Na technique (Green and Newgrain, 1979) and 'double-isotope' technique (Holleman *et al.*, 1975), respectively.

8.2 Methods

8.2.1 Experimental Animals

Measurements of milk intake were made using five female ringtail possums and their young maintained in captivity (see Chapter 2). Details of the animals and the sampling regime are given in Table 8.1. An additional three females and their offspring (also maintained in captivity) were used when comparing the absolute growth rate of the young with maternal body mass.

8.2.2 Estimation of Milk Intake

Pouch Young

While the young were in the pouch, milk intake was estimated by the ^{22}Na turnover technique of Green and Newgrain (1979) as modified by Green (pers. comm). Young were removed from the female (as in Chapter 7), weighed to the nearest 0.1g on a Mettler PE 3600 balance and then injected intraperitoneally with 50 μl of NaCl containing 20 $\mu\text{Ci/ml}$ of ^{22}Na . Prior to injection, the syringe used was calibrated by mass with distilled water (as in Chapter 6). The young were then placed in glass jars lined with tissues coated in vaseline within a humid incubator at 35°C. The mothers were weighed to the nearest 10g using a Pesola spring balance and then retained in cotton bags. Mother and young were kept separate for 4-5 hours to allow for isotope equilibration within the exchangeable sodium pool of the young, and to allow for milk accumulation in the mammary glands.

After this period the young was again weighed before a lateral tail vein was located and pricked for subsequent blood collection in Microcap capillary tubes. A 2 μl whole blood sample was washed out into 2mls of deionised water in a plastic vial and used for ^{22}Na determination by atomic absorption spectrophotometry (Varian, Model 1000). A second blood sample (10 μl) was washed out into 100 μl of

TABLE 8.1 Milk intake measurement details.

Mother	Suckling	Year	Period of Lactation (Days)	Technique Used*
F1	I	1987	60 - 81	N
			81 - 103	N
			103 - 124	N
			134 - 141	D/T
			152 - 157	D/T
F1	H	1987	(as I)	(as I)
F5	L	1987	59 - 80	N
			80 - 102	N
			102 - 123	N
			133 - 140	D/T
			151 - 157	D/T
F5	M	1987	(as I)	(as I)
F4	O	1987	59 - 79	N
			79 - 106	N
			106 - 118	N
			134 - 140	D/T
			153 - 158	D/T
F7	P	1987	68 - 101	N
			101 - 122	N
			147 - 153	D/T
			170 - 175	D/T
F6	K	1987	68 - 96	N
F6	J	1987	(as K)	(as K)

*Where N = ^{22}Na technique (Green and Newgrain 1979) and D/T = deuterium and tritium method of Holleman *et. al.* (1975).

water in a scintillation vial and then evaporated to dryness at 60°C. Subsequently 3ml of scintillation cocktail (PCS, Amersham) was added to each vial before counting in a liquid scintillation spectrometer (Searle, Isocap 300). A standard dilution of the ^{22}Na injection solution was prepared (i.e., 50 μl of ^{22}Na was made up to 50ml with distilled water) and analysed for ^{22}Na activity as described above. A sample of the distilled water used in the preparation of the standard was also analysed for ^{22}Na activity (blank).

Milk samples were collected from the mother, following the procedure described in Chapter 7. Then 5 μl of the milk was added to 2mls of deionised water for measurement of the sodium content by atomic absorption spectrophotometry (Varian Model 1000). The young were then returned to the mother and the procedure was repeated, without reinjection, at weekly intervals. At three weeks, when the level of ^{22}Na in blood samples became low, a blood sample was collected from the young to determine residual isotope levels after which the young were reinjected with more isotope.

Milk intake was not estimated in young that were less than 8 weeks of age because of the problems involved in collecting sufficient whole blood samples from pouch young less than 10g. The tail scars that remained after blood collection on hairless pouch young, either on the right hand side or left hand side of the tail, were used to distinguish between individual young in the pouch.

Calculations:-

Sodium influxes were derived from changes in the specific activity of ^{22}Na in the blood between bleedings. The following calculations were carried out:

(a) Exchangeable Sodium Pool Size (ES)

$$= \frac{\text{Injected counts}}{\text{Specific activity } (^{22}\text{Na}) \text{ in blood}}$$

Where injected counts = isotope standard counts - blank counts, and where specific activity (SA) = counts/ μmole of sodium.

(b) Sodium Influx (mMol)

$$= \frac{\log_n (Na1 \times ES1 / Na2 \times ES2)(ES2 - ES1)}{\log_n (ES2 / ES1)} + (ES2 - ES1)$$

Where Na1 and Na2 are initial and final specific ^{22}Na activities and ES1, ES2 are exchangeable sodium values for two consecutive blood samplings.

(c) Milk Intake (ml)

$$= \frac{\text{Sodium influx (mMol)}}{\text{Mean milk Na over sampling period (mMol/ml)}}$$

Back Young

Sodium intake from sources other than milk (ie. foliage) precludes the use of the ^{22}Na technique after the young have emerged from the pouch. All of the young had permanently vacated the pouch by 18 weeks post partum, therefore, the method used to measure milk intake by 'back-young' was that of Holleman *et al.*, (1975) as modified by Dove and Cork (1989).

The 'back young' were separated from their mothers and a sample of whole blood was taken from the tail vein of the offspring and from an ear vein of the mothers to assay for background levels of the isotopes. Occasionally the tail vein of the young was difficult to locate so in some young, blood was collected from the infra-orbital sinus. The mothers were then injected intraperitoneally with 0.5ml tritiated water (20 MBq) and the young were similarly injected with 1ml of deuterium oxide (98% atoms excess). Three hours were allowed for the isotopes to equilibrate in the body water pool (see Chapter 5). After this period the mothers and young were weighed and samples of blood were collected (at least 100 μl), as above. Milk was also collected from the female as described in Chapter 7 and used to confirm that the specific activity of tritium was the same as that of water in the blood.

The whole blood samples and milk samples were frozen (-20°C) and transported to the laboratory. Here the water from whole blood samples and milk samples was extracted by vacuum sublimation (Vaughan and Boling, 1961) and

tritium specific activity was determined by liquid scintillation spectrometry. The blood water samples were diluted 2 μ l to 1ml and deuterium levels in the body water were measured in an isotope ratio mass spectrometer. Standard dilutions of the injected isotopes were prepared (i.e., 1ml of tritiated water was made up to 500ml with distilled water and 50 μ l of deuteriated water was made up to 100ml with distilled water). These standard dilutions and the distilled water were analysed for isotopes as described above. The water content of the milk was measured as in Chapter 7.

After the final blood sample had been collected the young were replaced with the mothers and returned to their cages. One female and her two sucklings were sampled, as described, for blood and milk each day for six days. When levels of tritiated water in the offspring were plotted over time it was found that peak levels of tritium in the offspring occurred at about 5 days after injection (Fig. 8.1). Therefore all measurement periods were 5 days long with a minimum of 1 week between periods. This allowed isotope levels to fall to acceptable background levels and minimised disturbance to the animal.

Calculations:-

Isotope dilution spaces and total water inflows of mothers and offspring were calculated as described by Dove and Freer (1979), with appropriate adjustment for the increasing pool size of the offspring ie;

1. Tritium (HTO) specific activity in mother at t_0 (α_0)

α_0 = Equilibrium specific activity of HTO - background counts (DPM/ml)

2. Fractional turnover rate in mother (K_a)

$$K_a = (\ln \alpha_0 - \ln \alpha_t) / t$$

where α_t = Specific activity of HTO at time t

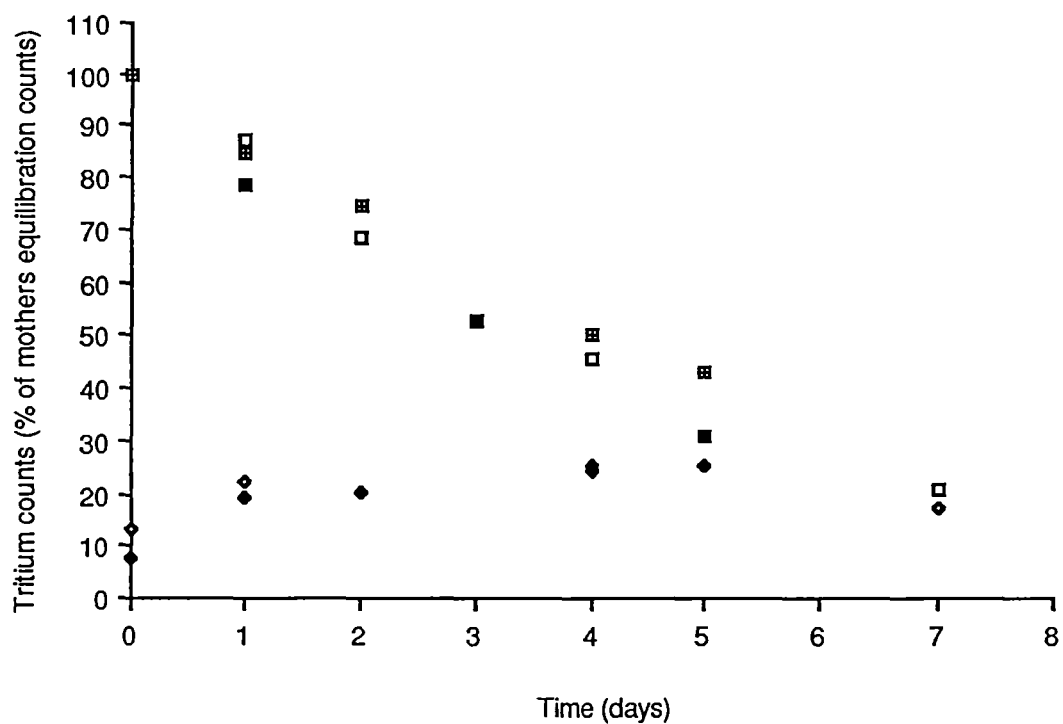


Figure 8.1 Tritium (HTO) activity, expressed as a percentage of the mothers equilibration counts, in three mothers and two sucklings, at intervals after equilibration.

- Mother F5
- Mother F5
- ▣ Mother F4
- ◆ Young L
- ◆ Young O

3. Pool size in young (V_o)

$$V_o(\text{ml}) = \frac{\text{Injected dose of D}_2\text{O (atm \%)}}{\text{Equilibrium D}_2\text{O concentration - background (atm \% / ml)}}$$

4. Fractional turnover rate in young (K_b)

$$K_b = \frac{\frac{V_t - V_o}{t} - \frac{\ln(C_t/C_o) - \ln(V_t/V_o)}{\ln(V_t/V_o)} + \frac{V_t - V_o}{t}}{V_o}$$

Where V_t = Estimated pool size at time t and,

C_o and C_t = concentrations of D_2O in the water of the young at equilibrium and at time t , respectively.

5. Amount of HTO accumulated in the young at time t (Q)

$$Q (\text{DPM}) = V_t \times \text{HTO}_t (\text{in young})$$

6. Milk water intake (MW) was calculated from the accumulation, in the offspring of HTO previously injected into the mother, as Holleman *et al.*, (1975) ie;

$$\text{MW}(\text{ml}) = \frac{Q(K_b - K_a)}{\alpha_0 (e^{-K_a t} - e^{-K_b t})}$$

7. Milk intake (M) is finally estimated as;

$$M = \text{MW} \times \frac{100}{\% \text{ water in milk}}$$

When young were reinjected some tritium was found to remain in the blood from the previous measurement period. This remaining tritium was estimated from the

following equation;

$$\text{HTO}_R = \frac{\text{D}_2\text{O}_T}{\text{D}_2\text{O}_E} \times \text{HTO}_E$$

Where; D_2O_T and D_2O_E are the concentrations of deuterium oxide in the young at time t and at equilibration respectively, HTO_E is tritium counts at equilibration and HTO_R is the tritium remaining in the young after a measurement interval of time t . This residual amount of tritium (HTO_R) was then subtracted from the counts obtained at each subsequent t , for the calculation of the amount of tritiated water accumulated in the young at time t (Q).

Insufficient equilibration blood was collected from very small pouch young in the early trials for measurement of isotope levels. Therefore, the missing equilibration concentration of deuterium in these young was estimated from a regression of pool size on body mass derived from other animals (Figure 8.2) i.e.,

$$\text{Pool size in young} = -28.260 + 0.7722 \text{ mass(g)}, r^2=0.907$$

Unknown equilibration pool size (V_0) was estimated from the known weight of the animal at equilibration and the missing equilibration concentration was subsequently estimated from;

$$\frac{\text{Injected dose of } \text{D}_2\text{O}}{V_0}$$

8.2.3 Energy Content of the Milk

The energy content of the milk was derived from the proximate composition of ringtail milk as determined in the previous chapter, and assumed energy values of 16.5, 24.6 and 38.1 kJ/g for milk carbohydrates, proteins and fats respectively (Ofstedal, 1984). Milk energy content from milk samples collected from ringtails in the wild (Chapter 7) was estimated in the same way.

8.2.4 Intake of Milk Components

Intakes of carbohydrate, protein, lipid, and gross energy were calculated by multiplying milk intake by the concentration of each milk component in milk samples taken at the time when intake was measured. The concentration of the carbohydrate, protein and lipid components in the milk samples were determined in

$$y = -28.260 + 0.7722x \quad r^2 = 0.907$$

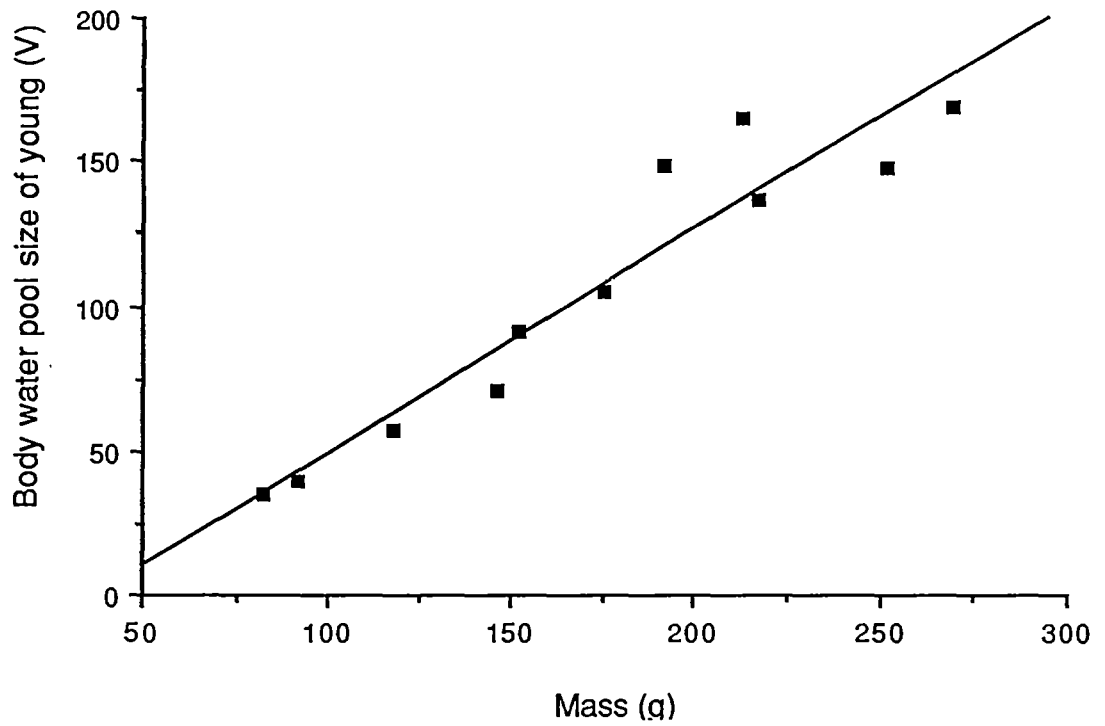


Figure 8.2 The relationship between body water pool size (ml) and body mass (g) in four suckling ringtail possums.

Chapter 7.

8.2.5 Statistical Methods

Means are presented ± 1 standard deviation. Where appropriate comparisons were made between two means using Student's t-tests (un-paired and two-tailed). Percentages were compared after arcsine transformation. Regression equations were calculated by least squares and are presented with the coefficient of determination, r^2 . The 0.05 level of probability was accepted as indicating statistical significance.

8.3 Results**8.3.1 Body Mass, Total Body Water (TBW) and Water Influx in Mothers.**

The change in body mass of the individual females measured throughout the lactation period are shown in Figure 8.3. Between 110 and 140 days of lactation the lactating females appear to lose mass. A Student's t-test showed a significant difference ($df=79$, $t=2.167$, $P<0.05$) between the masses of the females whilst they suckled pouch young (0-120 days of lactation) and the masses of the females after the young had finally left the pouch (120 - 220 days of lactation, see Fig. 8.3). The mean female mass for these two periods was 1020 ± 59 g and 990 ± 37 g respectively. Mean mass for the females over the whole period of lactation was 1007 ± 49 g ($n=85$).

The total body water space (TBW) of the mothers during late lactation (Phase 3), determined from HTO dilution showed no significant change over a 3 week period (Table 8.2). Mean TBW was 786.6 ± 35.3 ml ($n=4$) and mean TBW expressed as a percentage of body mass at equilibration was 78.16 ± 3.75 ($n=4$). The mean water influx estimated for the mothers between 133-158 days of lactation was 177.01 ± 38.3 ml/day ($n = 4$) or 177 ± 39.8 ml.kg⁻¹.d⁻¹ ($n = 4$) (Table 8.2). Values ranged from 126.5 ml/day for one female, at 137 days of lactation to 259.3 ml/day in another female, at 152 days of lactation.

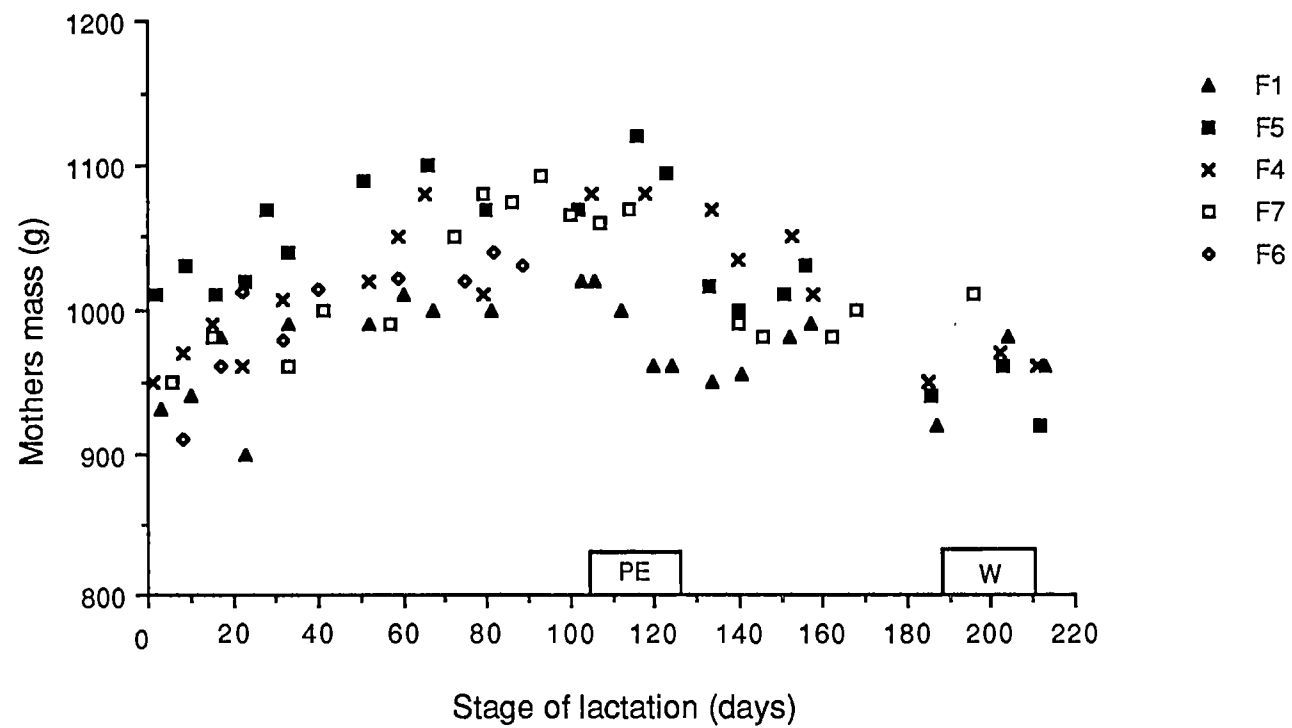


Figure 8.3 Changes in the masses of lactating females (in captivity), throughout lactation (n = 84)

TABLE 8.2 Changes in body mass total body water (TBW) and water influx rates in lactating ringtail possums.

Animal	Period of lactation (days)	Body mass# (g)	TBW space		Water turnover	
			(ml)	(%)*	(ml/d)	(ml.kg ⁻¹ .d ⁻¹)
F1	132 - 141	952	719	75.7	166.48	174.9
	152 - 157	985	759	77.4	219.60	222.9
F5	133 - 140	1018	838	82.6	182.40	179.2
	151 - 154	1013	752	74.4	259.30	255.9
F4	134 - 140	1052	792	74.0	126.50	120.3
	153 - 158	1030	785	74.8	138.30	134.3
F7	140 - 146	985	824	83.2	161.78	164.2
Mean (± s.d.)		1005 (± 33.3)	781 (± 41.6)	77.45 (± 3.88)	179.19 (± 46.5)	178.8 (± 44.5)

#= mean mass over measurement period, *= percentage of body mass.

8.3.2 Exchangeable Sodium, Isotope Dilution Space and Water Influx in the Young.

The masses and exchangeable sodium pool sizes (ES) of ringtail possum pouch young are given in Table 8.3. A linear relationship was found between the absolute ES and mass of the sucklings (Figure 8.4)i.e.;

$$\text{ES (mmol)} = 0.53012 + 0.0557 \text{ Mass(g)}, r = 0.9664, (n=13)$$

Table 8.4 summarises the body mass, deuterium oxide space (D_2O) and water influx of the young after they have emerged from the pouch ('back-young'). The body water pool of the 'back-young', expressed as a percentage of body mass averaged $60.83 \pm 12.29\%$ ($n = 6$) ranging from 45.57% to 77.5%. Mean absolute water influx by these young was 16.17 ± 5.28 ml/day ($n = 6$) and mean mass specific water influx was 124.8 ± 38.7 ml.kg⁻¹.d⁻¹ ($n = 6$)(Table 8.4).

8.3.3 Milk Intake Rates

Figure 8.5a and 8.5b show the mean daily rates of milk consumption by young between 10 weeks and 23 weeks of age. Values for weeks 10-18 and values for weeks 20-30 of lactation were estimates using the ²²Na technique and 'double-isotope' technique, respectively. Absolute milk consumption rates were low between 10 and 13 weeks of age, with the young consuming around 2.36 ml/day (Figure 8.5a). However mass specific milk intake rates by four young were relatively high at 10 and 11 weeks of age (i.e., 141.8 ml/kg.d, Figure 8.5b) before dropping to 81.6 ml/kg.d at 12 weeks of age. After 13 weeks post-partum, both absolute and mass specific milk consumption rates increased. The most rapid rate of increase in absolute milk intake was from 6 ml/day at week 15 post partum to 16ml/day at week 18, covering the period of lactation when the young emerge from the pouch. Milk intake was maximal (i.e., around 23.8 ml/day or 184.5 ml/kg.d) at 18 - 23 weeks (Figures 8.5 and 8.6).

Milk intake rates for individual animals increased exponentially with age and the degree of between animal variation increased with age (Fig. 8.6a). If milk intake

TABLE 8.3 Changes in mass and mass-specific exchangeable sodium pool of ringtail possum pouch-young.

Suckling	Stage of lactation (days)	Mass (g)	ES (mM/Kg)	Na in milk ($\mu\text{mol/ml}$)
I	103	39.8	69.11	14
	113	57.3	65.05	14
	120	72.5	63.11	17
	124	78.4	62.56	17
H	103	40.2	68.98	14
	113	57.2	65.07	14
	120	74.0	62.96	17
	124	79.9	62.43	17
L	102	51.3	66.13	13
	109	67.7	63.63	19.3
	116	91.8	61.57	14.5
	123	107.8	60.72	18
M	102	49.5	66.51	13
	109	64.7	63.98	19
	116	87.1	61.86	14
	123	108.4	60.69	18
O	105	56.8	65.13	13
	118	88.5	61.78	16
P	65	16.4	88.04	27
	72	18.8	84.01	28
	79	19.7	82.70	25
	86	28.2	74.59	21
	93	37.3	70.01	23
	93	36.5	70.32	23
	100	47.2	67.82	18
	107	66.1	63.82	19
K	114	111.8	60.54	15
	68	16.3	88.39	29
	75	19.7	82.67	28
	82	24.0	77.82	24
	89	30.4	73.23	23
J	96	38.3	69.64	18
	68	17.4	86.28	29
	75	19.3	83.26	28
	82	25.1	76.93	24
	89	30.2	73.35	23
	96	36.2	70.74	18

TABLE 8.4 Changes in the body weight, deuterium oxide (D₂O) space and water kinetics in ringtail sucklings.

Suckling	Period of lactation (days)	Body mass* (g)	D ₂ O space		Water inflow	
			(ml)	(%)**	(ml/day)	(ml.kg ⁻¹ .d ⁻¹)
I	134 - 141	85.76	39.0	45.57	6.33	73.80
	152 - 157	128.16	57.0	54.70	9.77	76.40
H	134 - 141	89.63	41.0	45.80	11.35	127.60
L	133 - 140	149.08	101.0	72.40	17.69	175.20
M	133 - 140	146.10	79.5	57.00	20.44	258.70
	151 - 154	234.63	136.0	62.64	16.21	69.27
O	134 - 140	160.25	91.0	59.80	21.49	134.30
	153 - 158	270.23	147.6	58.90	21.21	78.55
P	140 - 146	202.35	148.5	77.50	20.29	100.44
Mean (± s.d.)			93.4 (± 43.4)	59.40 (±10.7)	16.08 (± 5.61)	121.58 (± 62.5)

* mean body mass over measurement period. ** D₂O space as a percentage of body mass at equilibration.

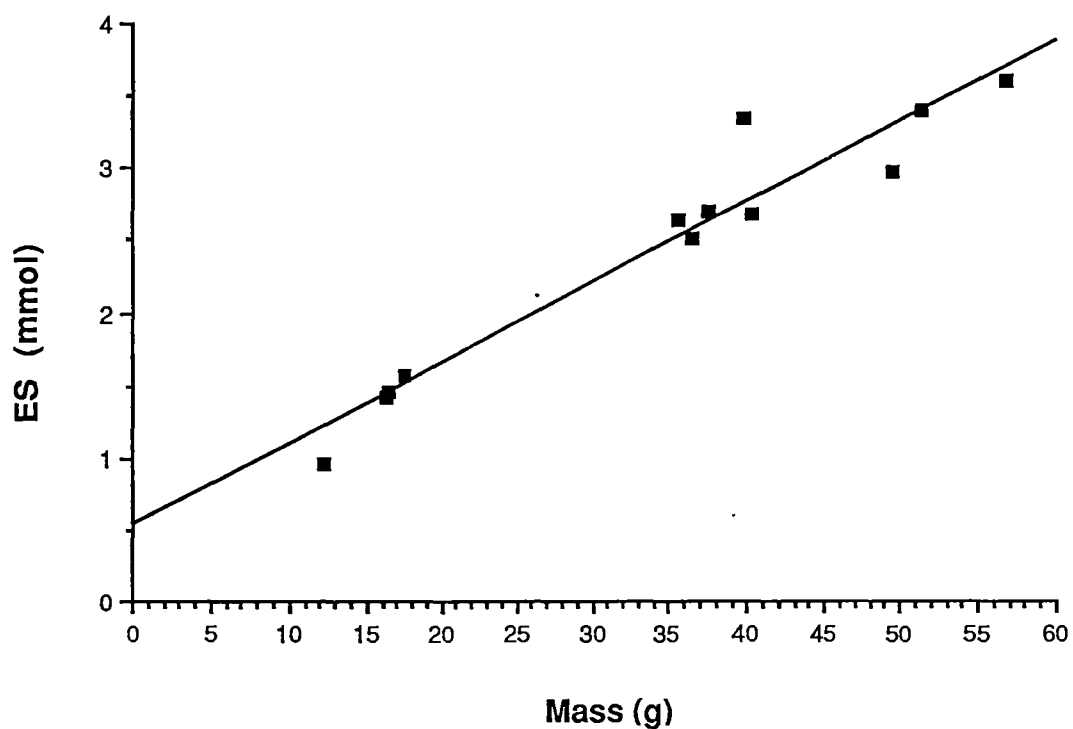


Figure 8.4 Linear relationship between absolute exchangeable pool size (ES) and mass of the suckling, i.e;
 $ES \text{ (mmol)} = 0.53012 + 0.0557 \text{ mass (g)}$, $r = 0.9664$
($n = 13$).

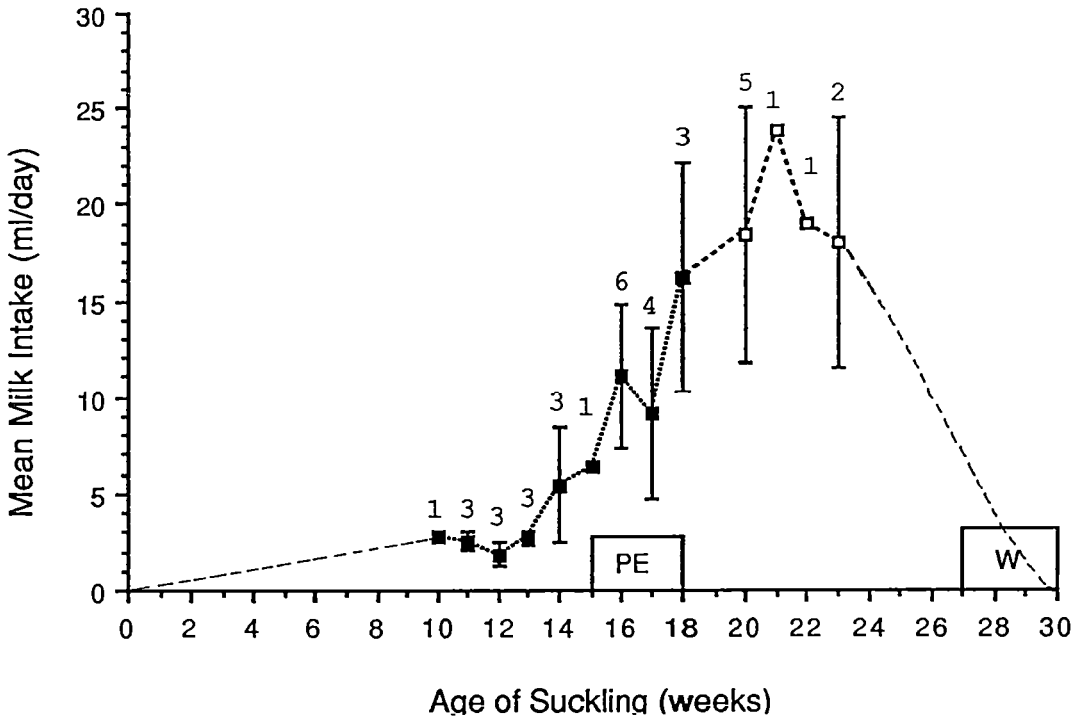
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Figure 8.5 Rates of (a) absolute milk consumption and (b) mass specific milk consumption by ringtail possum young between 10 and 23 weeks of age. Values given are means \pm sd. N for both (a) and (b) is given above error bars in (a). Closed squares are values estimated by the ^{22}Na technique (Green and Newgrain, 1979). Open squares are values estimated using the 'double-isotope' technique (Holleman *et. al.*, 1975).

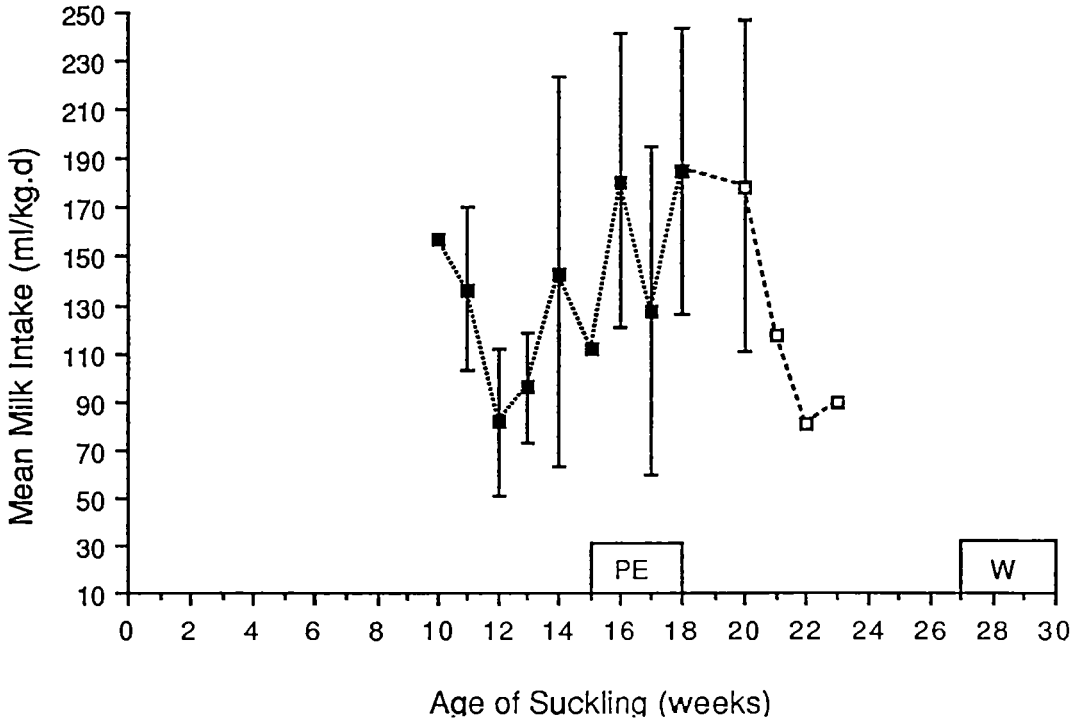
Dotted line on (a) indicates assumed changes in rates of milk intake by the young over the periods of lactation when measurements of milk intake were not made (see text, Cork and Dove 1988).

PE = pouch emergence, W = final weaning, (see Chapter 3).

(a)



(b)



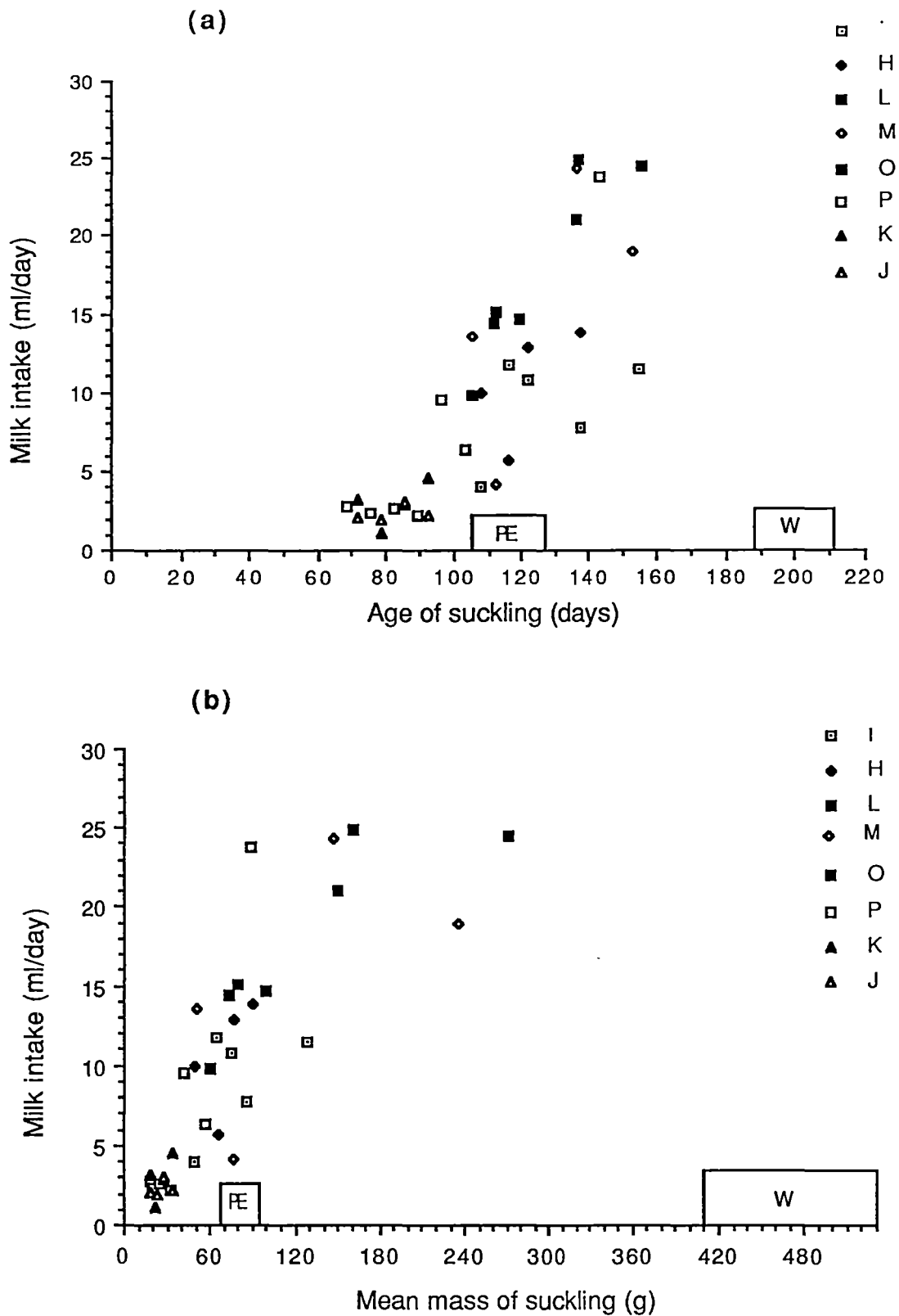


Figure 8.6 Rates of milk intake by eight young at (a) different ages and (b) of different mass. Identity of individual young is given in the legend.
PE = pouch emergence, W = final weaning.

rates are plotted against mass, ignoring age, the degree of scatter is reduced (Figure 8.6b). Milk intake rates (ml/day) by pouch young appeared to increase linearly in relation to body mass. A regression of daily milk intake plotted against body mass prior to pouch emergence illustrates the significant linear increase in milk intake ($df=25$, $t=7.035$, $P<0.05$) by pouch young (Fig. 8.7).

i.e., Milk intake (ml/day) = $-0.9771 + 0.1638 \text{ Mass (g)}$, ($r^2 = 0.6731$, $n=27$).

Figures 8.8(a-f) illustrate the intake of milk solids components (carbohydrate, protein and fat) with respect to age and mass of the young throughout lactation. The peak rate of fat intake by the young was lower than that of the other milk components (i.e., 1.0 g/day and around 1.5g/day, respectively). The rate of intake for each milk component appears to reach a plateau when the young reach 180g (Figure 8.8 a,c and e). However, further data is needed to fully describe the trend of milk intake rates during the later stages of lactation.

8.3.4 Energy Content of the Milk

The mean energy content of the milk of captive *P. peregrinus* changes throughout lactation (Figure 8.9a). Milk energy gradually increased from 3.3 kJ/ml at 5 weeks of lactation to 5.0 kJ/ml at 15 weeks of lactation. After 15 weeks, milk energy dropped rapidly to about 2.8 kJ/ml over the period when the young were emerging from the pouch. A slight rise in milk energy to 3.5 kJ/ml occurred towards the end of lactation between week 24 and week 30 post partum.

Figure 8.9b illustrates the relative contributions of milk components to the total energy content of milk collected from captive animals. Between week 5 and week 15 of lactation carbohydrate makes up 55% of milk energy before dropping rapidly between week 15 and week 18. At pouch emergence carbohydrate contributes 30% to milk energy and its contribution continues to decline until weaning (Figure 8.9b). The contribution made by protein to milk energy was lower than carbohydrate during pouch life but increased rapidly during the stage when the young emerge from the pouch and represented 50% of milk energy during late lactation (Figure 8.9b). The lipid contribution to milk energy remained low throughout lactation between 20% and 25%.

The energy content of individual milk samples collected from lactating ringtail

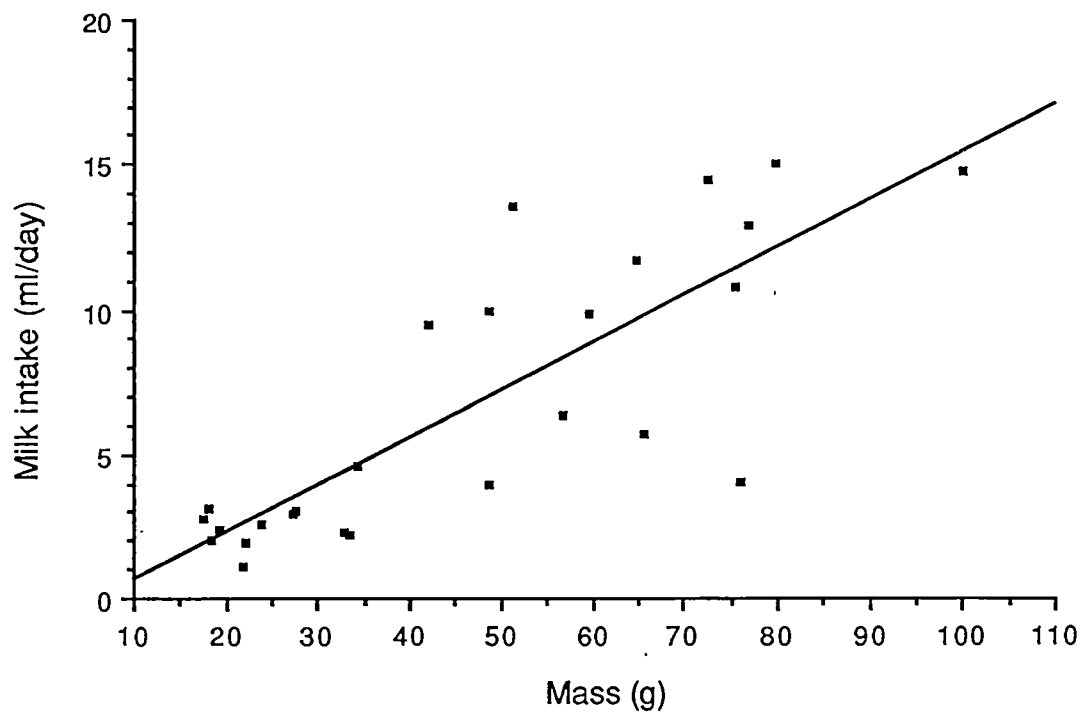
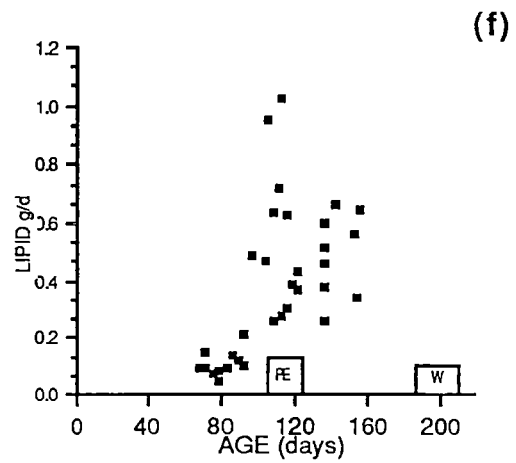
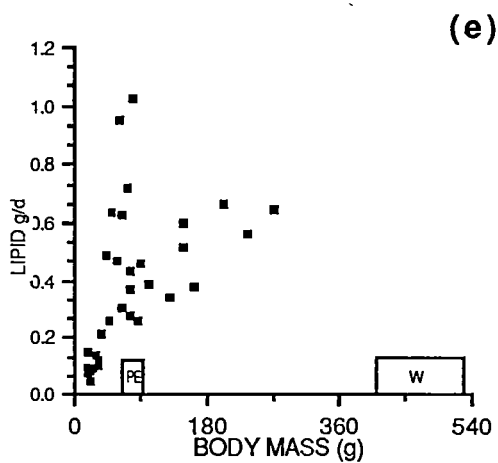
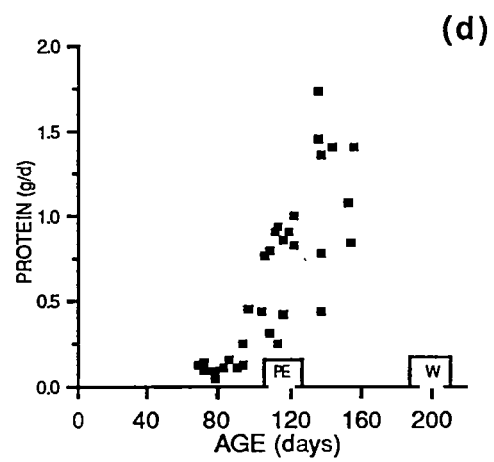
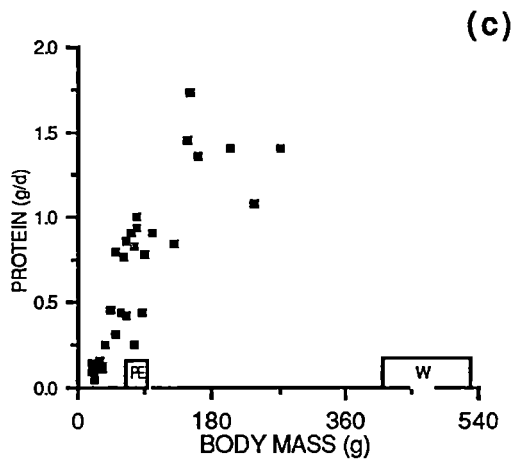
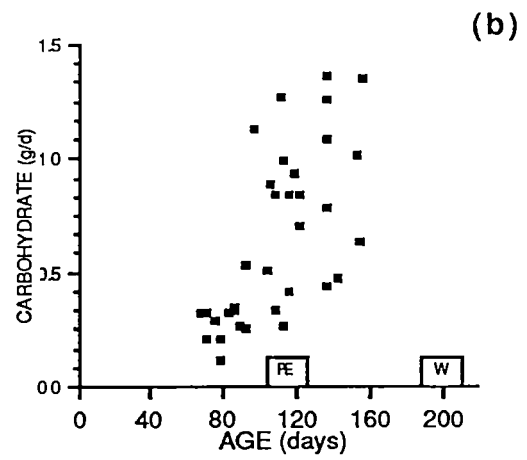
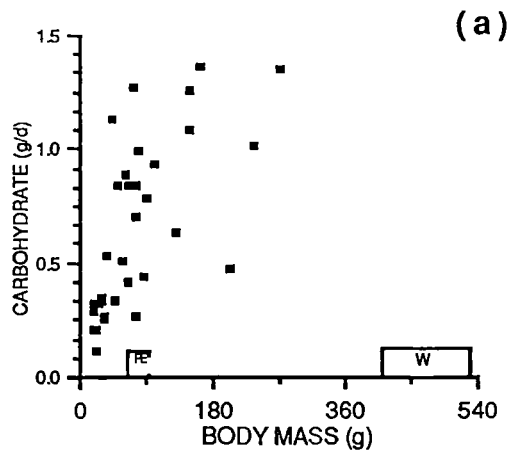
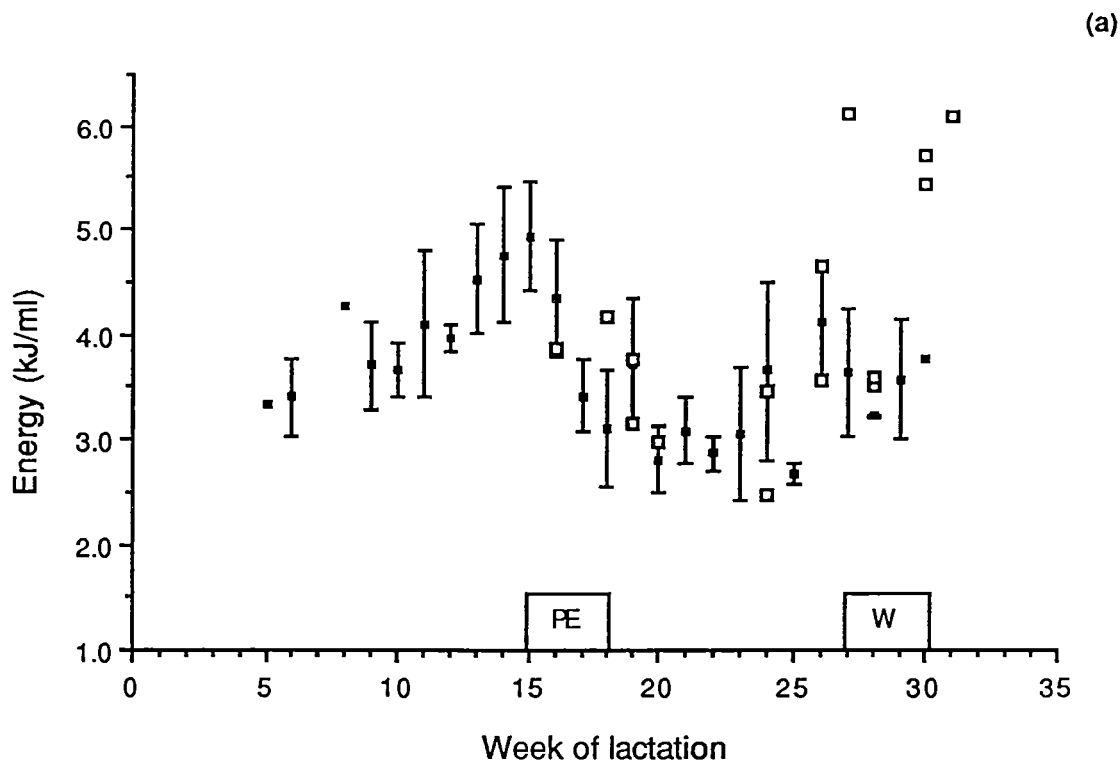


Figure 8.7 Rates of milk consumption by ringtail possum young of different body masses during pouch life.
Milk intake (ml/day) = $-0.9771 + 0.1638 \text{ Mass (g)}$
($r = 0.8204$, $n = 27$)

Figure 8.8 Intakes of milk carbohydrate (a,b), protein (c,d) and lipid (e,f) by ringtail sucklings in relation to their body masses (a,c,e) or ages (b,d,f). Individual values are given.
PE = Pouch emergence W = final weaning.





(b)

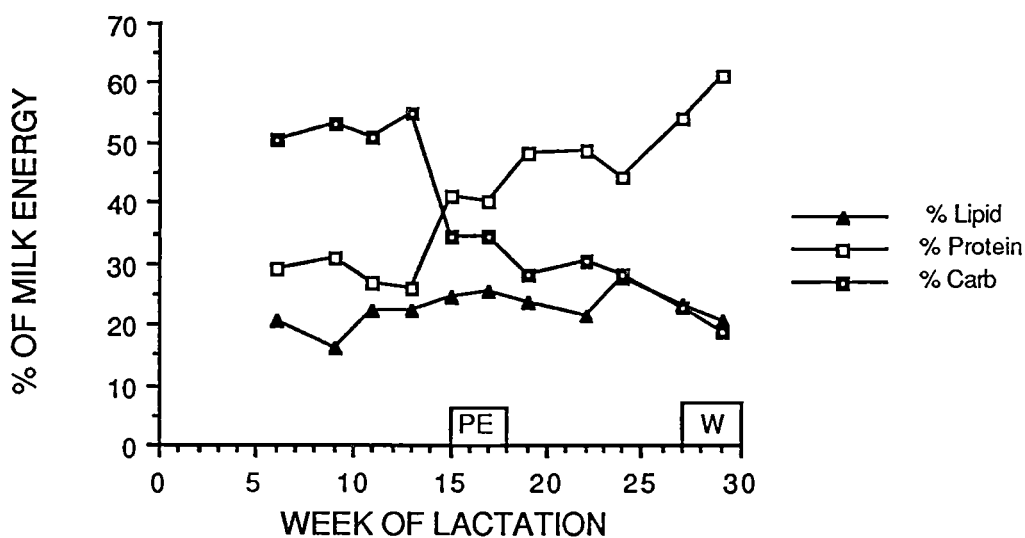


Figure 8.9 (a) Energy content of milk collected from captive ringtail possums (■, means \pm s.d, $n = 122$) and, wild ringtail possums (□, individual values, $n = 15$). (b) Mean contribution of carbohydrate, protein and lipid to total energy of milk collected from captive ringtail possums. PE = pouch emergence, W = final weaning.

possums in the wild are presented in Figure 8.9a. Although the number of samples obtained from wild animals are low they appear to show the same pattern of change in milk energy content as the samples collected from captive animals. The mean energy content of milk collected from captive animals and wild animals between week 15 and week 31 of lactation was 3.5 ± 1.058 kJ/ml ($n = 75$) and 4.2 ± 1.139 kJ/ml ($n = 16$), respectively. However, this difference was not significant ($df = 89$, $t = 2.454$, $P > 0.05$).

Figure 8.10 illustrates the percentage contribution made by milk components (protein, fat and carbohydrate) to the energy content of milk collected from wild animals compared with the values obtained for milk from captive animals between week 15 and week 31 of lactation. No significant difference was found between the percentage contribution made by carbohydrate to the energy content of the milk collected from wild animals and that of milk from captive animals ($df = 89$, $t = 1.797$, $P > 0.05$). The contribution made by the lipid fraction to milk energy was significantly higher in milk from wild animals compared to captive milk ($df = 89$, $t = 9.009$, $P < 0.05$, respectively). However, at the same time the contribution made by milk protein in wild animals was lower than that found in the milk of captive animals ($df = 89$, $t = 8.81$, $P < 0.05$).

8.3.5 Milk Energy Intake and Peak Milk Energy Yield.

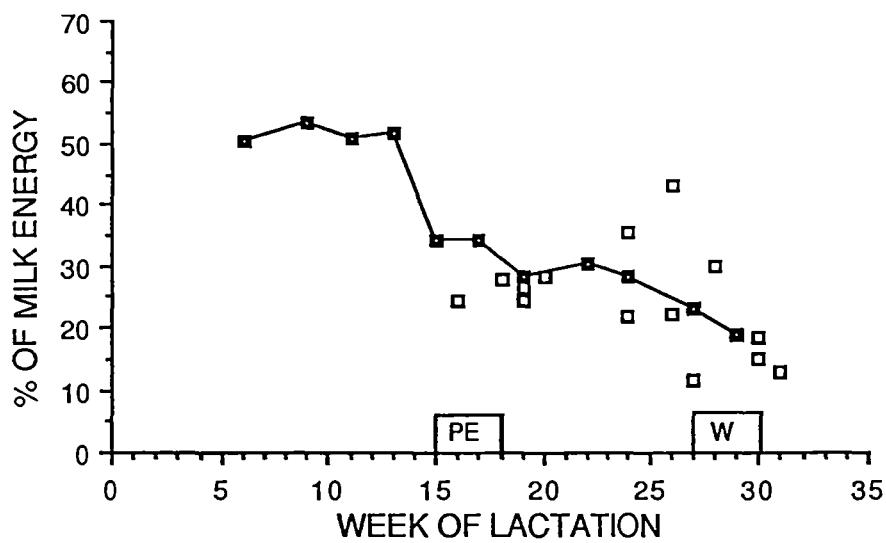
Milk energy intake rates (kJ/day) for sucklings between 10 and 23 weeks of age are shown in Figure 8.11. Peak milk energy intake was estimated to be 77.8 kJ/day at 22 weeks of age. Assuming that milk production equalled the measured milk consumption, peak milk energy yield by a female suckling two young was estimated to be 155.52 kJ ($154.4 \text{ kJ/kg}^{0.75}$) at 22 weeks of lactation. When expressed as the energy output per litter metabolic mass (Ofstedal, 1984) peak milk energy yield for a female with two young is $124 \text{ kcal/kg}^{0.83}$.

8.3.6 Crude Growth Efficiency (CGE)

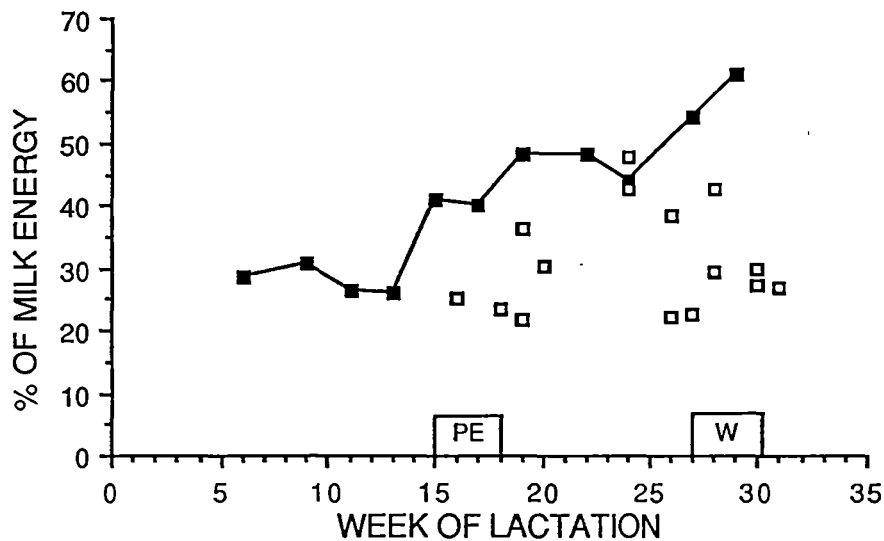
The increase in body mass (g) for each ml of milk consumed (CGE) and milk consumption rates (ml/day) for suckling ringtails over the period of lactation examined are summarised in Table 8.5. Between 10 and 12 weeks post partum 0.19

Figure 8.10 Relative contribution of **(a)** carbohydrate, **(b)** protein and **(c)** lipid to milk energy (all expressed as a percentage of total milk energy) in individual milk samples from wild ringtail possums compared with values obtained for captive animals.

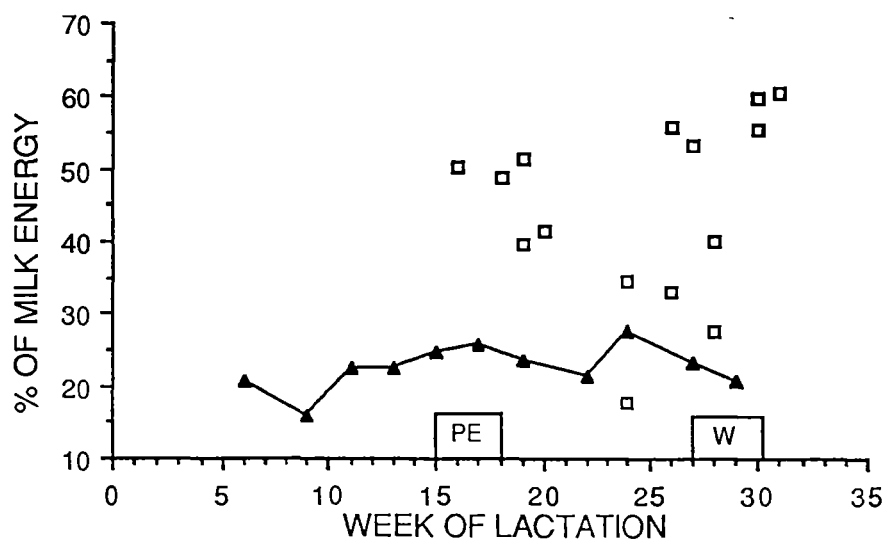
(a)



(b)



(c)



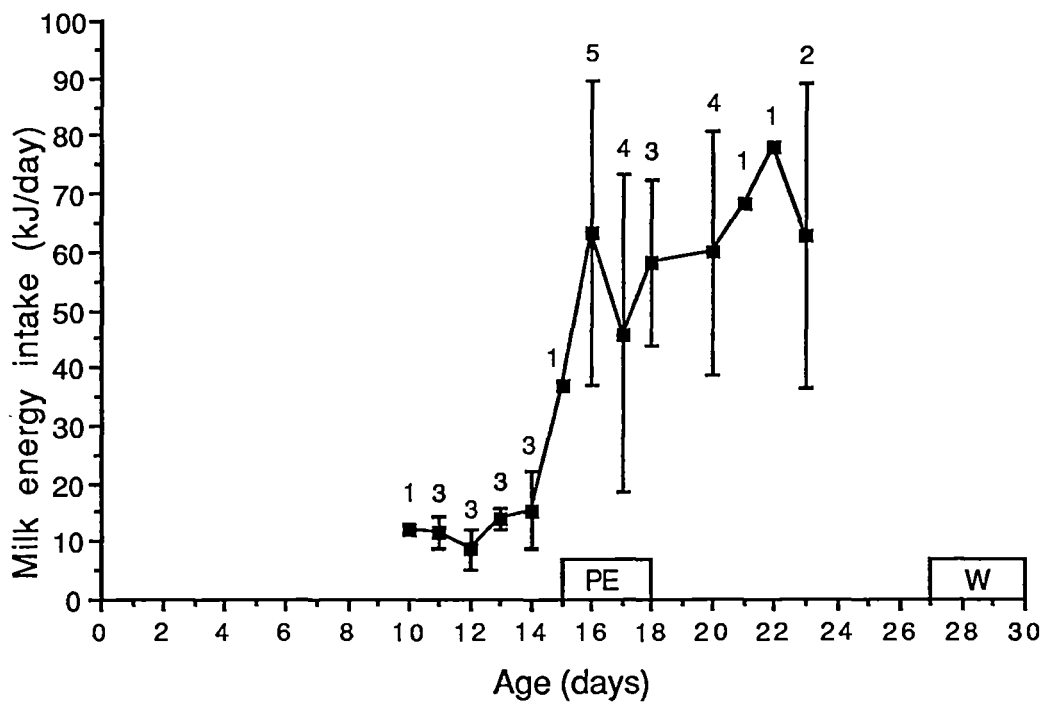


Figure 8.11 Rates of energy intake (kJ/day) by ringtail possum sucklings between 10 and 23 weeks of lactation. Values given are means \pm sd. N is given above error bars.
PE = pouch emergence, W = final weaning (see Chapter 3)

TABLE 8.5 Milk consumption rates and Crude Growth Efficiencies (CGE) in suckling ringtail possums.

Age (weeks)	Mean Mass (g)	Daily Milk Intake (ml)	Daily Mass Increase (g) (g/ml milk)*	
10 - 12	19.8	2.36 (± 0.20 , n=3)	0.44 (± 0.15 , n=3)	0.19 (± 0.07 , n=3)
12 - 14	31.0	3.36 (± 0.51 , n=2)	0.91 (± 0.10 , n=2)	0.27 (± 0.01 , n=2)
15 - 17	64.9	10.39 (± 3.57 , n=6)	2.68 (± 0.97 , n=6)	0.28 (± 0.12 , n=6)
20 - 21	138.9	19.26 (± 6.96 , n=6)	2.88 (± 1.36 , n=6)	0.15 (± 0.04 , n=6)
22 - 23	211.0	18.31 (± 6.55 , n=3)	9.30 (± 3.16 , n=3)	0.51 (± 0.04 , n=3)

* CGE, crude growth efficiency, represents the body mass increment for each gram (or millilitre) of milk consumed.

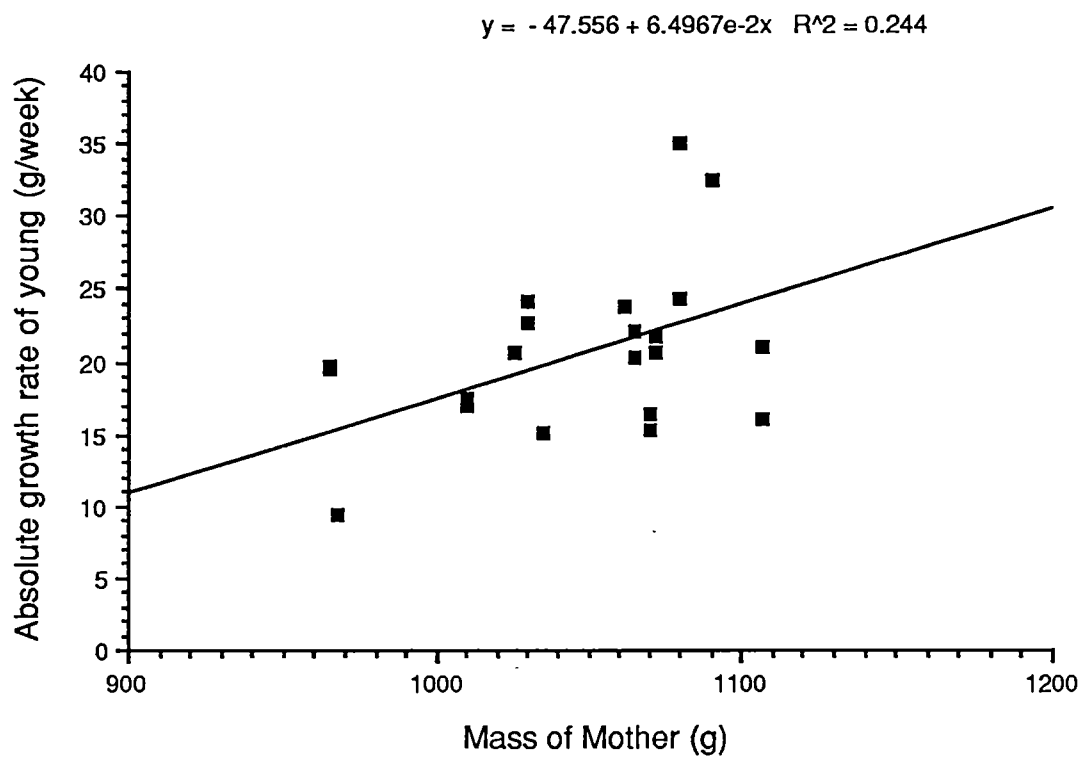


Figure 8.12 The relationship between absolute growth rate of sucklings (g/week) and mean maternal mass during weeks 16-18 of lactation.

± 0.15 grams ($n=3$) of body mass were accrued for each ml. of milk consumed. CGE increased to around 0.28 g/ml milk consumed between 12 and 17 weeks post partum. This increase corresponds with the rapid rise in body mass of sucklings between week 14 and 17 of lactation (see also Chapter 3). The increased CGE coincides with the increase in milk solids, particularly carbohydrate at this time (see Chapter 7).

CGE dropped to 0.15 g/ml between week 20 and 21 post partum. This drop coincides with a drop in milk solids after the young have emerged from the pouch (Chapter 7). However a relatively high level of CGE (0.51 g/ml) was estimated for three animals measured between week 22 and 23 of lactation. This increase in CGE corresponded with a peak in daily mass increase (Table 8.5).

The absolute growth rates of 11 sucklings during weeks 16-18 of lactation were compared with the body masses of their mothers (Figure 8.12). The absolute growth rates of the sucklings (AGR) and maternal body mass were found to be significantly related ($df= 42$, $t = 2.538$, $P<0.05$), i.e.;

Mothers mass (g) = $-47.556 + 0.065$ AGR (g/week), $r^2=0.244$, $n = 44$.

8.4 Discussion

A validation trial conducted by Green *et al.*, (1988) on tammar wallaby, *Macropus eugenii*, sucklings established a high correlation between ^{22}Na estimated sodium influx and the actual sodium influx from the milk. However they found that the ^{22}Na method overestimated milk intake in *Macropus eugenii* by about 10%. A similar validation trial was not attempted in this study due to the logistical problems in obtaining ringtail possum young for hand rearing and the immense practical problems involved in hand rearing pouch young of this species. Therefore, it should be noted here that the rates of milk intake by ringtail possum pouch young measured using the ^{22}Na technique, may be slight overestimates. Similarly, since *Macropus eugenii* is the only marsupial species for which this technique has been validated, the measurements of milk intake rates by other marsupial species, studied so far, may also be overestimates. Logistical problems also prevented validation of the use of the 'double-isotope' technique to estimate milk intake by the ringtail possum 'back-young' in this study. Nevertheless, validation experiments by Holleman *et al.*, (1975) and Wright and Wolff (1976) showed that reliable measurements of

milk intake can be achieved with this technique. Cork and Dove (1989) found a good agreement between estimates of milk intake by *Macropus eugenii* sucklings made using the ^{22}Na technique (Green *et al.*, 1988) and estimates of milk intake made at the same stage of lactation using the 'double-isotope' method which justifies combination of the two techniques to describe milk intake throughout lactation in marsupial species.

In this study, since all the young had fully emerged from the pouch when the 'double-isotope' method was used, isotope recycling via excreta consumption by the mother was considered to be minimal. However isotope recycling may also occur after the young have left the pouch, whilst the mother grooms the young in the nest. Nevertheless, Dove *et al.*, (1989) examined this problem in *Macropus eugenii* and demonstrated that after the young have left the pouch even 100% recycling of isotope via excreta consumption resulted in only small errors in the estimate of milk intake. The recycled isotope is extensively diluted in the mothers pool, which is much larger than either the offspring's pool or milk intake.

The 'double-isotope' method used in this study to estimate milk intake by the young during Phase 3 of lactation provided additional information on the total body water (TBW) and water influx of the mothers and sucklings. The mean proportion of body mass made up by TBW (78.16%) and mean water influx rates ($177 \text{ ml.kg}^{-1}.\text{d}^{-1}$) measured for the mothers suckling 'back-young' were similar to those obtained for lactating ringtail possums in the wild (i.e., 80% and $156.7 \text{ ml.kg}^{-1}.\text{d}^{-1}$, see Chapter 6). Mean TBW was not estimated in wild ringtail possum sucklings. However, the values of TBW (estimated as D_2O dilution space) as a proportion of body weight obtained for ringtail sucklings in this study (45.7%-77.5%) were lower than those reported by Dove and Cork (1989) for *Macropus eugenii* (73.6%-79.3%).

The period of time allowed for the deuterium isotope to equilibrate with the body water pool of the young, in the present study, was an hour longer than that used in similar studies (Dove and Freer, 1979; Dove, 1988; Dove and Cork, 1989). However, if animals are sampled after complete equilibration has occurred overestimates of total body water usually result due to a loss in body mass (Kunz and Nagy, 1988; Gales, 1989). Therefore the relatively lower TBW values obtained for the suckling ringtail possums in this study were not considered to be a result of inaccurate equilibration times. The lowest values for D_2O dilution space expressed

as a proportion of body weight (45.7%-54.7%) were obtained for two sucklings, H and I, whose absolute growth rates (g/day) were lower than those of other young of the same age (Chapter 3). No significant difference was found between the rates of milk intake by these young compared with the other sucklings at the same stages of lactation (df=33, $t=-0.023$, $P>0.05$). However, they had significantly lower water inflow rates compared with those of other sucklings (df=8, $t=-3.215$, $P<0.05$). Therefore, young H and I may have been dehydrated which would explain their low TBW's.

Ignoring the TBW's of these young the mean value of TBW for the remaining young was $65 \pm 8.3\%$ (n=6) which is still lower than that recorded for *Macropus eugenii* sucklings (75.3%). Studies have shown that % total body water is inversely proportional to the amount of fat in an animal's body (Pace and Rathburn, 1945; Searle, 1970; Green and Eberhard, 1983). This suggests that, in general, the young ringtail possum has a higher body fat content than *Macropus eugenii* young at an equivalent stage of development.

Rates of milk intake by the ringtail possum sucklings changed throughout lactation, i.e., low intake during the early stages of lactation rising substantially during pouch emergence and then reaching a peak after the young had left the pouch. This pattern was similar to that described for *Macropus eugenii* (Cork and Dove, 1989; Green *et al.*, 1988). An increase in milk intake rates during pouch life, measured using the ^{22}Na technique, have also been noted in *Trichosurus vulpecula* (Cowan, pers. comm.); *Potorous tridactylus* (Crowley, 1984; Smolenski and Rose, 1988) and *Isoodon macrourus* (Merchant and Libke, pers. comm.).

The mean values obtained for absolute milk intake (ml/day) by ringtail sucklings between 10 and 24 weeks post partum were generally lower than those obtained for *Macropus eugenii* at equivalent stages of lactation (Green *et al.*, 1988; Dove and Cork, 1989). When expressed as the intake of milk solids per $\text{kg}^{0.75}$ (i.e., correcting for the metabolic weight of the young; Blaxter, 1961) the rate of solids intake by ringtail possum young are still lower than in *Macropus eugenii* young (Table 8.6). However, the rate of milk solids intake by ringtail possum young is similar to that noted for *Trichosurus vulpecula* (Cowan, pers. comm.). The highest rates of milk solids intake ($\text{ml/kg}^{0.75}$) recorded for a marsupial are those of *Bettongia gaimardi* during late pouch life (Table 8.6). This reflects the fast growth

TABLE 8.6 Milk intake, intake of milk solids related to the offspring's metabolic weight and crude growth efficiencies (CGE) during pouch life in some marsupial species.
MP = Middle pouch life. LP = Late pouch life.

Species and stage of lactation	Daily milk intake (ml.day ⁻¹)	Intake of milk solids (g.Kg ^{-0.75})	CGE Daily mass increase (g/ml)	Source
<i>Macropus eugenii</i>				
MP	4.72	18	0.21	Greenet.al., 1988
LP	51.22	24	0.33	
<i>Potorous tridactylus</i>				
MP	3.65	10	0.14	Crowley, 1984; Smolenski and Rose, 1988
LP	17.70	-	0.20	
<i>Bettongia gaimardi</i>				
MP	-	-	-	Smolenski 1986
LP	80.80	30.6	0.22	
<i>Trichosurus vulpecula</i>				
MP	2.07	11	0.22	Cowan pers.comm.
LP	31.58	22	0.30	
<i>Pseudocheirus peregrinus</i>				
MP	2.36	11	0.19	This study
LP	10.39	19	0.28	

rate and short duration of lactation exhibited by this species (Smolenski, 1986; Smolenski and Rose, 1988).

The changes in milk consumption rates reflect the changes in body mass of the suckling. As the rate of milk intake increased during lactation the weekly mass increment also increases (Chapter 3). The most pronounced increase in rate of milk intake occurs over the period when the young are leaving the pouch, between week 15 and 17 post partum. This corresponds with a significant increase in absolute growth rate (g/week) and the highest instantaneous relative growth rate (Chapter 3). The absolute growth rate (g/week) of ringtail possum young during week 16-18 of lactation was closely related to maternal body mass, i.e., a faster growth rate was correlated with a heavier mother. The growth rate of other mammalian offspring during the nursing period has also been positively correlated with maternal body mass (e.g., *Peromyscus leucopus*, Myers and Masters, 1983; *Trichosurus vulpecula*, Bell, 1981 and *Tachyglossus aculeatus*, Green *et al.*, 1985). Green *et al.*, (1988) proposed that heavier mothers produce more milk and hence faster growing young.

The body mass that accrued from each ml of milk consumed by the ringtail possum (CGE) increased from week 10 to week 17 post-partum, reaching values of 0.28g/ml during pouch emergence. A similar increase in CGE has been noted for other marsupial species (e.g., *Macropus eugenii*, Green *et al.*, 1988 and *Potorous tridactylus*, Crowley, 1984) and reflects the increase in milk solids during pouch life (Chapter 7). Immediately after the ringtail possum young have left the pouch, estimates of CGE declined which corresponded with the drop in mass increment and instantaneous growth rate noted at this stage (Chapter 3). Several authors consider the period of pouch emergence and immediately afterwards to be a particularly energy demanding stage for the suckling because of the increased energy requirement for maintaining body temperature homeostasis and independent mobility (Green *et al.*, 1980, Oftedal, 1980). The decline in CGE is followed by a dramatic increase to 0.51 around week 22 which is also reflected in a significant peak in mass increment (Chapter 3). This increase only partially represents the mass increment derived from milk since the young are eating solid food at this time. Hence it represents an apparent increase in CGE as solid food intake was not measured.

Although the rates of solids intake ($\text{g} \cdot \text{kg}^{-0.75}$) for the ringtail are generally

lower than estimates obtained for *Macropus eugenii* (Green *et al.*, 1988) and other marsupials studied (Crowley, 1984; Smolenski, 1986; Smolenski and Rose, 1988) the crude growth efficiency at various stages of lactation are generally comparable to those of other marsupials (Table 8.6). Therefore, the relatively long duration of lactation and low absolute growth rate of the ringtail when compared with other marsupial species (Chapter 3) is due to a limited rate of supply of milk energy and not to inefficient conversion of milk energy. Green (1984) and Green *et al.*, (1988) note that the changing pattern of mammary gland size and mass, in *Macropus eugenii* paralleled changes in milk consumption rates and growth rates of the young. They suggested that this represented regulation of growth rates in the young by means of limited milk production. Similarly, the changes in milk consumption rates by ringtail sucklings over the period studied reflect changes in size of the mammary glands (Chapter 7).

The energy content of ringtail possum milk is generally lower than the energy content of milk (kJ/ml) from other marsupial species studied so far (Table 8.7). In general, milk carbohydrate is the major source of energy for the marsupial suckling during early pouch life (Table 8.7). While the ringtail sucklings are leaving the pouch the rate of energy intake increases rapidly with milk protein as the primary energy source. There is also a slight increase in the contribution of milk lipid energy to the total energy content of the milk at this stage. Although, the contribution to total milk energy made by the lipid component in Phase 3 milk collected from wild ringtail possums is substantially higher than that of captive animals it is still lower than recorded for milks of other marsupial species. Furthermore, the total energy content of Phase 3 milk from both captive and wild ringtail possums is lower than that of milks from other marsupial species (Table 8.7) in which a large increase in milk lipid concentration results in a major increase in the energy content of milk after the young have left the pouch. Therefore, after the ringtail possum young have left the pouch with its relatively constant environment and readily available nourishment, they are sucking milk with a relatively low energy content. Nevertheless, since the rate of milk intake by the ringtail possum young increases as they leave the pouch and for a few weeks after, their rate of energy intake increases.

Oftedal (1980) suggests that the proportion of milk energy provided by protein is a useful index of the resources available for infant growth, as protein is essential for tissue growth and development. Immediately after the young have left

TABLE 8.7 Concentration of milk energy and the contribution of milk components to milk energy in some marsupial species.

Species	Stage of Lactation	Energy Content (KJ/ml)	% Contribution to milk energy			Reference
			Prot.	Carb.	Lipid	
<i>Macropus Eugenii</i>	E	2.5	26	44	30	Green <i>et al.</i> (1980), Green <i>et al.</i> (1988)
	M	5.0	30	30	40	
	L	11.5	29	9	62	
<i>Potorous Tridactylus</i>	E	2.5	34	47	13	Crowley <i>et al.</i> (1988)
	M	8.0	24	16	60	
	L	12.0	24	5	67	
<i>Bettongia gaimardi</i>	E	3.0	43	42	15	Smolenski and Rose (1988)
	M	6.0	38	39	23	
	L	10.0	30	10	60	
<i>Trichosurus vulpecula</i>	E	4.0	30	40	30	Cowan (1988)
	M	7.0	27	23	50	
	L	6.0	21	28	51	
<i>Isoodon macrourus</i>	E	2.4	50	27	23	Green and Merchant (1988)
	M	7.9	32	16	52	
	L	11.5	21	11	68	
<i>Dasyurus viverrinus</i>	E	4.0	36	36	28	Green <i>et al.</i> (1987)
	M	5.4	36	15	49	
	L	9.8	15	5	80	
<i>Pseudocheirus peregrinus</i>	E	3.3	30	50	20	This study
	M	5.0	40	35	25	
	L	3.7	50	30	20	
	L*	4.2*	32*	25*	43*	

E = Phase 2 milk; M = period covering pouch emergence; L = Phase 3 milk ; * milk from wild *Pseudocheirus peregrinus*.

the pouch, during the phase of adjustment to an adult leaf diet (described as digestive metamorphosis by Janssens and Ternouth 1987), they spend a lot of time in the nest with litter mates and/or the mother (Chapter 4; Thompson and Owen 1964). When they do leave the nest, the young forage close to their mother and often travel on her back. These behavioural strategies probably reduce the energy demands on the young for physiological activities such as thermoregulation and for locomotion. Therefore, during this early stage of independence the young may be adapted to a low requirement for energy for free-living. The increased contribution of protein to the total milk energy intake by the young, at this stage, may therefore primarily reflect the growth requirements of the young.

Rose (1987) has suggested that additional energy requirements, such as energy required for locomotion and thermoregulation, partition available energy away from growth after the marsupial young has emerged from the pouch. It seems reasonable to assume that these additional energy requirements would be greater for free-living sucklings compared with those living in captivity. For this reason and the fact that the Phase 3 milk from free-living animals had a lower protein content and a lower protein contribution to milk energy, a lower relative growth rate was expected for wild young. However, no difference was found between the growth rates of field sucklings and captive sucklings (Chapter 3). Lipid makes a higher contribution to the total energy content of Phase 3 milk from wild animals compared with that of captive animals. Therefore, this may supply the milk energy necessary to satisfy the possible additional energy requirements of free-living young thus reducing energy partitioning away from growth.

Milk yield by most mammalian species increases to a peak level and then declines over a period of time. When combined with information on the composition of milk, peak milk yield provides an estimate of peak energy output which has been used to compare milk energy yields by different species (Ofstedal, 1984). The estimated peak milk energy output ($154.8 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$) for the ringtail possum is at the lower end of the range of values ($280\text{--}586 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$) obtained for other herbivorous species for which data are available (Ofstedal, 1985) including that measured for the marsupial, *Macropus eugenii* (i.e., $207 \text{ kJ.kg}^{-0.75}.\text{d}^{-1}$; Cork and Dove, 1989). This low peak energy yield is comparable with those recorded for two primate species ($146.4 - 163.2 \text{ kJ/kg}^{0.75}.\text{d}^{-1}$; Ofstedal, 1984). The ratio of litter metabolic mass to maternal metabolic mass ($\text{kg}^{0.83}/\text{kg}^{0.75}$) may be used to predict

the peak milk energy output of a particular species (Oftedal, 1984). The predicted peak milk energy yield for a ringtail possum suckling two young and a metabolic mass ratio of 0.53 (i.e., $2 \times 0.235^{0.83} / 1.010^{0.75}$), is $120 \text{ kcal/kg}^{0.75} \cdot \text{d}^{-1}$ (Oftedal, 1984). This value is greater than the measured energy output. As with some primate species (Oftedal, 1984), low peak energy yields per litter metabolic mass in the ringtail possum are correlated with low rates of growth in developing young (Chapter 3).

The use of peak milk energy yields to compare the milk energy outputs of different species is not particularly satisfactory since the length of lactation and the ratio of peak yield to total yield varies between species (Oftedal, 1984). For example, although the ringtail possum has a peak milk energy yield which is lower than many other mammalian species, its long period of lactation (Chapter 3) may result in a similar total milk energy yield to other species. Information on the energy content of milk and milk yield at each point on the lactation curve is required to obtain an estimate of total milk energy yield.

The steady decline in size of the ringtail mammary gland after week 22 of lactation (Chapter 7) may coincide with a decrease in milk intake rate by the young until they are fully weaned, as noted for *Macropus eugenii* (Green, 1984; Green *et al.*, 1988; Dove and Cork, 1989). A decrease in sucking frequency by the young (personal observation) over this period further supports this suggestion. Therefore, it was assumed that the milk intake rate by the ringtail possum suckling decreased exponentially from week 23 to final weaning (week 30) and increased linearly from birth to week 10 of lactation (see dotted line, Figure 8.5a). Furthermore, daily milk intake rates within a given week were assumed to be equal to the mean daily intake rate for that particular week. Thus milk yield (intake) was estimated for the weeks of lactation not measured. This was then combined with measured values of milk energy content and milk energy yield, enabling estimation of the total milk energy yield by a ringtail possum (mean body mass of $1006.8 \pm 48.9 \text{ kg}$, $n = 85$) suckling two young for 30 weeks, i.e., 11 MJ/kg . Information on total milk energy yield is only available for a few herbivorous eutherian species (Oftedal, 1985). However, comparisons involving eutherian and marsupial species are complicated by the comparatively longer time invested in lactation by marsupial species. Nevertheless one recent study has detailed the total milk energy yield by a marsupial herbivore *Macropus eugenii* (Dove and Cork, 1989; Cork and Dove 1989). Martin (1984)

stresses the importance of body size in any broad comparisons of milk energy yield between species. Cork and Dove (1989) argue that since daily milk energy yield appears to scale to (maternal mass)^{0.75} amongst mammals (Martin 1984; Oftedal, 1984, 1985) and physiological time scales to (maternal mass)^{0.25}, total milk energy yield should scale to (maternal mass)¹. Therefore, the value estimated for the ringtail possum was similar than the value obtained for the 5kg herbivorous marsupial *Macropus eugenii* (12.6 MJ/kg; Cork and Dove, 1989).

In summary, in addition to a dilute milk with a low energy content the peak energy yield by the ringtail possum appears to be comparatively low. However, mass-specific overall milk energy yield by the ringtail possum was similar to that of another herbivorous marsupial, which suggests a spreading of the milk energy drain on the mother over time. This lactational strategy correlates with the slow rate of growth by the sucklings (Chapter 3) which does not appear to be related to an inefficient conversion of milk but rather a limited rate of milk production by the mother. Russell (1982) notes that the actual growth rate of the young in many marsupial species varies throughout the period of growth, never reaching the maximum rate possible. Therefore, restriction of growth rates by the regulation of milk supply and milk composition (Chapter 7) by the mother may be general for all marsupial species (Green, 1984). Observations on transfer of marsupial pouch young between species or within species at different stages of lactation (Merchant and Sharman, 1966; Rose, 1984) supports this idea i.e., the transfer of small *Macropus eugenii* pouch young to larger mammary glands or vice versa results in increased mass gain by the small young and emaciation or death of the large young (Findlay, 1982b).

Although the changes in milk composition of marsupial species appear to have a common pattern there are minor differences between the species (Merchant *et al.*, 1989). These differences may extend to the quantities of milk produced by different species and may be correlated with lifestyle and environmental influences (Merchant and Libke, 1988). For example, the low overall milk energy yield by the ringtail possum may be related to its energetically conservative lifestyle (Chapter 4 and 6) which appears to be a result of a low rate of energy intake dictated by its leaf diet (Leigh and Smythe, 1978; McNab 1978; McNab 1980).

CHAPTER 9

GENERAL DISCUSSION

The efficiencies and absolute rates of energy use by mammals are highly variable (McNab, 1986a). This variability appears to be due to many factors, including body size and food habits (McNab, 1978; Nagy, 1987). Previous studies on the energetics and activities of species which feed on leaves have led several authors to suggest that a characteristic of arboreal folivores is a conservative lifestyle, in energetic terms (Eisenberg, 1978; McNab, 1978; Hume *et al.*, 1984). The daily rates of energy expenditure (Chapter 6) and activity patterns (Chapter 4) of non-breeding adult ringtail possums appear to support this hypothesis. The relatively low energy requirement for metabolism by the ringtail possum reduces the amount of food required and hence may be adaptive to a leaf diet low in available energy and relatively high in toxic chemicals. Energy for reproduction must be acquired in excess of an animal's basic energy requirements (i.e., maintenance, activity and thermoregulation). The amount of energy that females allocated to reproduction, and rate at which this energy was allocated, are consistent with the ringtail possums apparently energy-conservative lifestyle.

9.1 Energy Allocation to Reproduction

Many behavioural studies suggest that the energetic cost of mating is high for males (Gittleman and Thompson, 1988). However, there was no apparent increase in the energy expenditure of the male ringtail possums during the mating season. In contrast, the daily metabolisable energy expenditure of female ringtail possums varied significantly according to their reproductive status. The measurements of metabolisable energy expenditure (Chapter 6) and milk energy output (Chapter 8) enabled calculation of the total energy allocated to lactation by the ringtail possum. This was then combined with measurements of time expended in lactation (Chapter 3) to provide annual energy and time profiles for a breeding female rearing two young (Figure. 9.1).

Without the burden of lactation, the annual energy expenditure of an adult female would be 213.4 MJ.year⁻¹ (Chapter 6). However, the annual energy

expenditure for a breeding female rearing two young (most common litter size) was $247.8 \text{ MJ}\cdot\text{year}^{-1}$ (Figure 9.1). Although other breeding activities i.e., mating and gestation, may also constitute an energy cost, lactation is obviously the most energetically expensive period for the female ringtail possum, with Phase 3 of lactation in particular accounting for 30% of the total yearly energy expenditure, during 24% of the time.

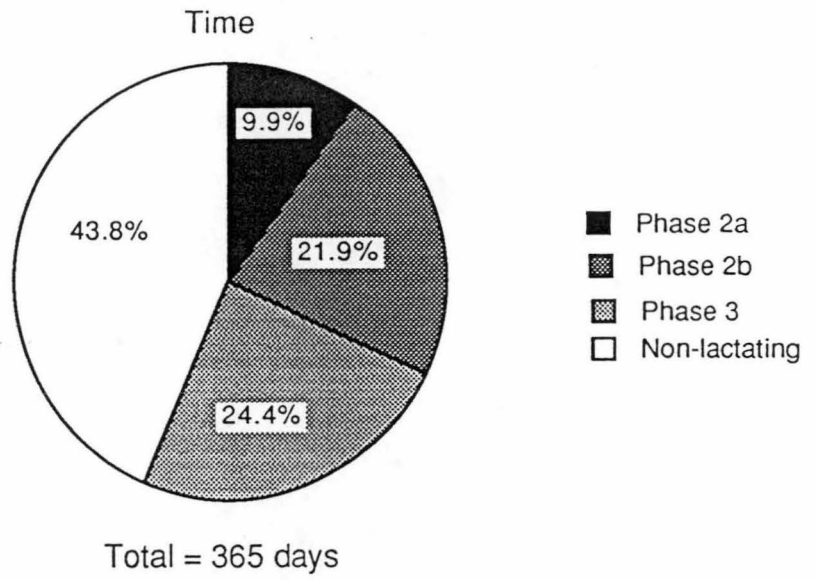
An animal may employ three basic strategies to achieve energy balance when faced with an increase in energy expenditure (as discussed by Racey and Speakman, 1987):

1. Increasing the quantity of digested energy i.e., increasing food consumption, digestive efficiency or reducing energy loss in urine and faeces (Glazier, 1985).
2. Utilising stored energy (Anderson and Fedak, 1987).
3. Compensating by reducing expenditure on some component of the energy budget.

The pattern of seasonal change in body mass of the male ringtail possum (Chapter 3) suggests that males utilise stored energy, in part, to meet the possible energy costs of mating. In addition compensatory allocation of energy may be important (Chapter 6).

In order to determine how a female meets the increased energy requirement of lactation a rudimentary daily energy budget was constructed (Table 9.1) based on the components highlighted in Figure 9.2. The energy required for metabolism includes energy for maintenance (e.g., resting, thermoregulation, activity and travel) and energy for synthesis of products (e.g., energy storage, somatic growth and milk). Two of the maintenance costs (thermoregulation and rest) were estimated from measurements of time expenditure (Chapter 4) and from measurements of rates of respiratory energy metabolism (Chapter 5, see Table 9.2). The tissue mass of reproducing females, as estimated from total body water (Chapter 6), changed throughout the annual cycle and reflected the pattern of change in body mass. The cumulative increase in tissue mass during the non-lactating, Phase 2a and Phase 2b stages of the females cycle was found to equal the loss in tissue mass by Phase 3 lactating females (Table 9.1). Some of this increase in tissue mass may be due to somatic growth (i.e. development of mammary glands) or the developing foetus in pregnant females which may be included in the non-lactating group. However, for simplicity, it was assumed to be due entirely to energy stored as fat. Thus the

(a)



(b)

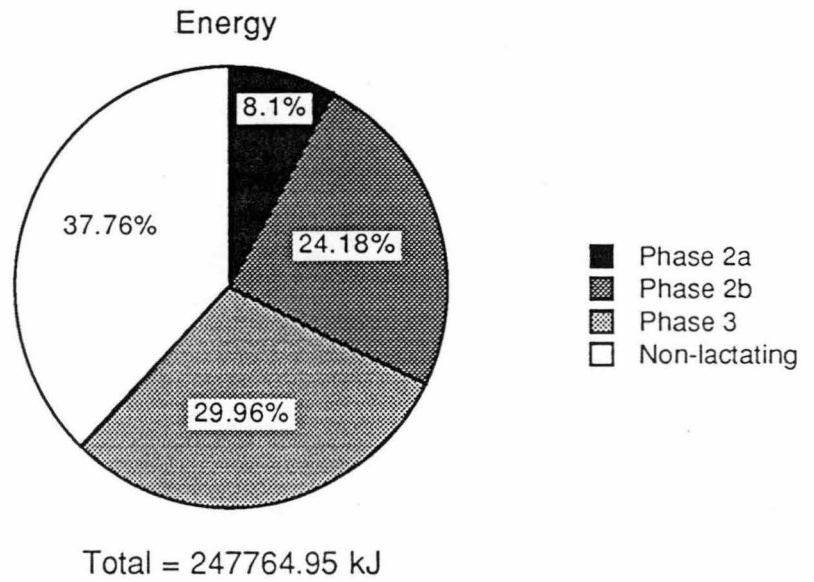


Figure 9.1 Annual (a) time and (b) energy profiles of a breeding female ringtail possum rearing two young. For explanation of Phases of lactation see Chapter 3.

Table 9.1 Daily energy requirement (respiratory energy metabolism plus chemical potential energy in products that may be synthesised) of adult female ringtail possums. (see Chapter 3 for explanation of the Phases of lactation)

	Non-lactating	Phase 2a	Phase 2b	Phase 3
Mean body mass (g) ^a	968.0	968.0	1059.0	993.0
Body tissue mass (g) ^b	232.2	288.2	300.0	203.4
Energy metabolism (kJ/d) ^c	584.7	550.6	698.1	759.2
Thermoregulation (kJ/d) ^d	66.4	77.9	84.4	56.1
(%)	11.4	14.1	12.1	7.4
Resting (kJ/d) ^e	188.9	181.0	200.4	197.0
(%)	32.3	32.9	28.7	26.0
Residual (c-d-e) [*] (kJ/d)	329.4	291.7	413.3	506.1
(%)	56.3	52.9	59.2	67.0
Stored energy (kJ/d) ^f	9.3	61.7	5.7	0.0
Exported milk energy (kJ/d) ^g	0.0	7.0	51.5	75.1
Total (c + f + g)	594.0	619.3	754.4	834.2
				757.5 ^{**}

a. Body mass of females from Chapter 6.

b. Body tissue mass for females in Chapter 6 estimated from total body mass - total body water estimates.

c. Total metabolisable energy measured using doubly-labelled water technique (Chapter 6).

d. Energy required for thermoregulation estimated from the net metabolic increase, at the mean minimum temperature for the appropriate months (NL = 12.2° C, Phase 2a = 9.0°C, Phase 2b = 6.7°C, Phase 3 = 10.6°C, Chapter 2,3), above thermoneutral zone resting metabolism (271.79 kJ.kg⁻¹.d⁻¹, Chapter 5) and the time each female group spent out of the nest at night (i.e., NL = 9.91 hrs, Phase 2a = 11.16 hrs, Phase 2b = 10.83 hrs, Phase 3 = 8.5 hrs, Chapter 4). The insulating nests used by the possums were assumed to maintain animals within their thermoneutral zone.

e. Energy required for resting estimated from the time each female group spent in nest plus the time spent out of the nest but stationary or grooming (i.e., NL = 17.3 hrs, Phase 2a = 16.5, Phase 2b = 16.7, Phase 3 = 17.3, Chapter 4) and the resting metabolic rate of ringtail possums at thermoneutrality (271.79 kJ.kg⁻¹.d⁻¹, Chapter 5).

f. The potential energy in synthesised somatic products. Estimated from the change in body tissue mass between female groups (i.e., NL = +37.3g, Phase 2a = + 56g, Phase 2b = + 11.6 g, Phase 3 = -105g) and assuming production consisted entirely of fat. Production in grams was converted to kJ using the factor 39.7 kJ/g (Kleiber, 1961)

g. The chemical potential energy in the milk exported to the young (see Chapter 8)

* Energy required for active behaviours (i.e., foraging, locomotion, interactions) and energy for synthesis of products stored (i.e fat) or exported (i.e milk).

* * Estimated total energy required minus metabolised stored energy.

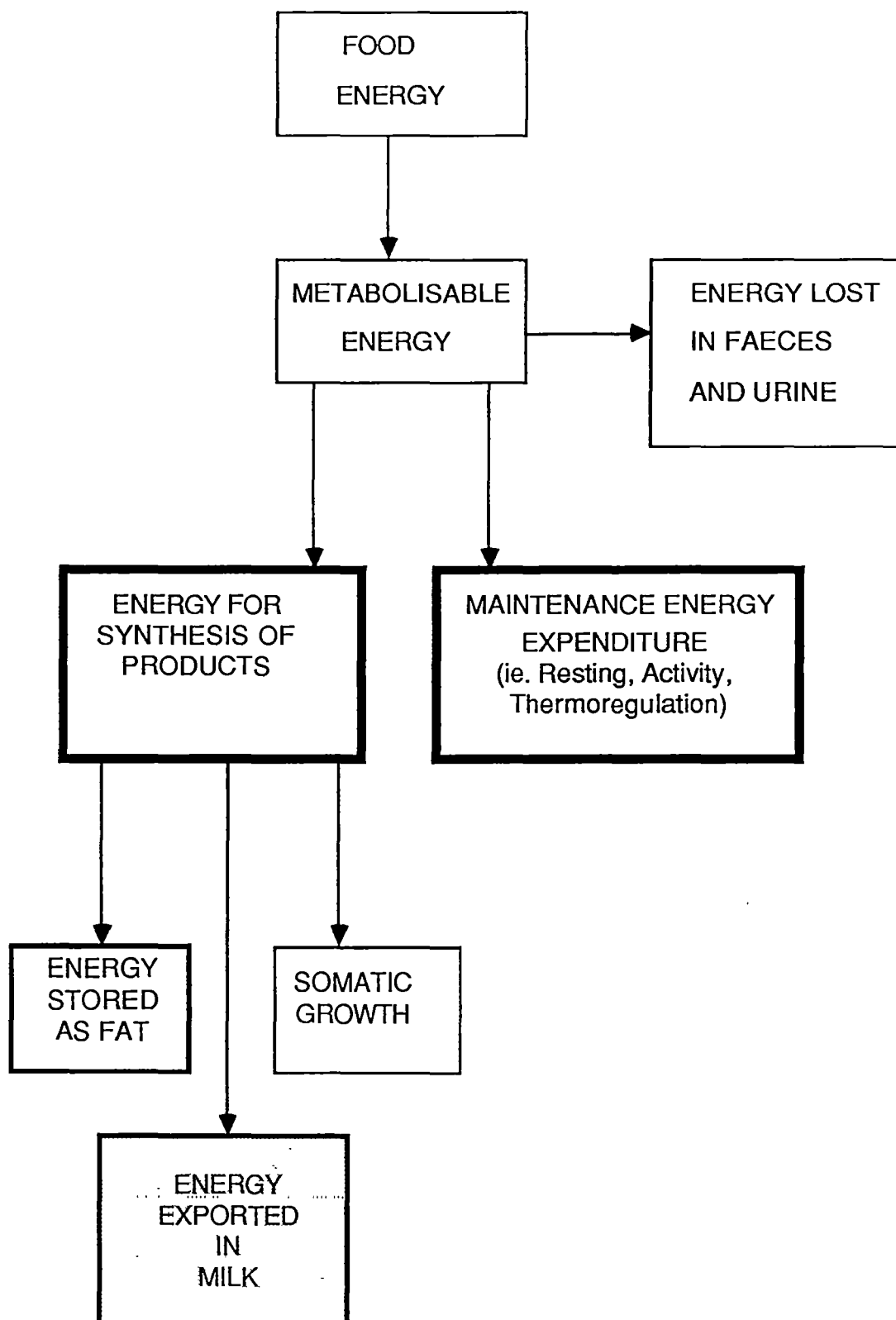


Figure 9.2 Components of a breeding females daily energy budget. Boxes outlined in bold indicate components measured using doubly-labelled water (Chapter 6). Shaded component was estimated from the milk energy content and the milk intake of the young (Chapters 7 and 8).

energy required by non-lactating, Phase 2a and Phase 2b females was assumed to be energy for respiratory metabolism plus the potential energy stored as body fat.

Comparison of the daily energy requirements of females at different stages of lactation, constructed in this way, suggests that the female ringtail possum maintains energy balance during lactation by employing the three strategies to varying degrees. For example, the energy required for Phase 3 of lactation is met by energy stored predominantly during Phase 2a of lactation and an increase in food energy intake (Table 9.1). The female appears to partially increase her food energy intake by increasing the time she spends feeding (Chapter 4). However, the rate at which a diet high in fibre can be processed may set an upper limit to the rate of energy intake. Nevertheless, the timing of late lactation coincides with the production of young leaves which have a higher available energy content than mature leaves (Chapter 3; Cork and Pahl, 1984; Pahl and Lee, 1988). Therefore, by consuming these leaves the female may increase her overall energy and nutrient intake. Furthermore, timing of late lactation to coincide with the warmer spring and summer months (Chapter 2) reduces the amount of energy required for thermoregulation.

The metabolisable energy cost of reproduction may be defined as the metabolisable energy requirements (e.g., for synthesis and secretion of milk) over and above non-reproductive maintenance requirements. The energy expended in lactation by the ringtail possum was assumed to approximate the energy allocated to reproduction since that expended in gestation, over three weeks, (Chapter 3) is likely to be negligible (Thompson and Nicoll, 1986). The total metabolisable energy expenditure of a female ringtail possum rearing two young is 236.8 MJ. year⁻¹ and the total metabolisable energy expenditure of a non-reproductive female is 213.4 MJ. year⁻¹ (calculated from the metabolisable energy figures in Table 9.1 multiplied by the appropriate time interval given in Figure 9.1). Assuming that total metabolisable energy requirements during reproduction (as opposed to daily energy requirements) scale to W^1 (Cork and Dove, 1989) the metabolisable energy cost of reproduction to the ringtail possum may therefore be calculated as 23.4 MJ/kg. This value is within the range of values available for the metabolisable energy requirements above maintenance of eutherian herbivorous mammal species (10.5 - 36.2 MJ/kg; Oftedal, 1985). It is also similar to that estimated for the marsupial herbivore, *Macropus eugenii* (20.9 MJ/kg; Cork and Dove, 1989). However, the peak daily energy requirement of the ringtail possum (759.2 kJ.d⁻¹, Table 9.1) was lower than the allometric prediction of Kirkwood (1983) for maximal metabolisable energy intake of a 993g mammal (1704.3 kJ.d⁻¹). Thus although the daily energy requirement during the most demanding phase of lactation appears to be lower than expected based on values for other mammal species, the total energy requirement for metabolism during reproduction is not.

However, the total annual energy commitment to reproduction includes both metabolised energy and the potential energy exported to the young via the milk.

This has not been measured for any other marsupial species and although measurements are available for a few eutherian species the variety of techniques used hampers comparisons. Nevertheless a study on free-living ground squirrels, *Spermophilus saturatus* found that the total annual energy allocated to reproduction by females rearing 2.7 young, estimated by a similar method to that used in this study, was 24% greater than the energy used by a non-lactating female (Kenagy, 1987). This was greater than the value of 16% obtained for a ringtail possum rearing two young (Chapter 6 and 8, see also Figure 9.1). Furthermore, the physiological reproductive effort value for the ringtail possum, estimated as the proportion of a breeding female's total annual energy budget that is used for reproduction (Randolph *et al.*, 1977), is lower than that estimated for cricetine rodent species (McClure, 1987) from food energy intake (i.e., 14% and 27-32%, respectively).

It may be suggested that this relatively low total allocation of energy to reproduction by the ringtail possum supports the hypothesis that energy transfer between mother and young is less efficient in marsupials than in other mammals (Lillegraven *et al.*, 1987). However, the metabolisable energy required for reproduction by the ringtail possum falls within the range for eutherians occupying a similar trophic group, as does that of another marsupial herbivore, *Macropus eugenii* (Cork and Dove, 1989). Therefore, the ringtail possums' relatively low overall energy expenditure in reproduction is a result of a low milk energy output. Since, the energy content of ringtail possum milk is lower than that of marsupial species in other trophic groups (Chapter 8), this low energetic investment in reproduction is not considered to be a marsupial characteristic but an adaptation to diet and lifestyle. Measurements of the energy required for reproduction by comparable folivorous eutherians (e.g., the sportive lemur, *Lepilemur leucopus*) would enable clarification.

9.2 Lactational Strategy

Information on the growth energetics in the ringtail possum derived from this study suggests that physiological constraints on the mother have the most influence on the lactational strategy employed, as in other marsupial species (Green, 1984; Crowley, 1984; Smolenski and Rose, 1988; Green *et al.*, 1989). In general, the

metabolic requirements of the female appear to influence the duration of lactation and growth of the suckling through the regulation of milk supply and milk composition (Chapter 8). The small differences in milk composition and milk production between marsupial species may be related to differences in diet and other lifestyle characteristics. For example, the ringtail possum has a long duration of lactation, dilute milk with a low energy content, and a low peak rate of milk production, relative to other marsupial species (Chapters 3,7,8).

The efficiency of milk secretion may be calculated as the proportion of metabolisable energy above maintenance that is secreted in the milk (Ofstedal, 1985). The net efficiency of milk secretion by the ringtail possum i.e., $10.97 \text{ MJ}/23.4 \text{ MJ} \times 100 = 47\%$, calculated for all lactation Phases combined, is lower than the range of values available for eutherian herbivorous species in which a low milk efficiency correlates with a low quality diet (Ofstedal, 1985). Therefore, the rate at which energy and nutrients can be expended in milk production by the ringtail possum may be constrained by characteristics of its leaf diet i.e., low protein intake and low rate of energy intake (Chapter 4; Smith and Lee, 1984). The ringtail possum, being the smallest folivorous marsupial, also has size related physiological constraints on the elevation of its food intake (Parra, 1978; Chapter 1). However, as the energy costs of lactation are spread over time, the peak energy and nutrient requirements of the ringtail possum are reduced.

The lactational strategy of the ringtail possum, therefore, is consistent with the energy-saving hypothesis (McNab, 1978; Eisenberg, 1978; Hume *et al.*, 1984). However, Eisenberg (1981) suggests that selection for a milk of high nutritive content is relaxed by some species as part of the positive selection for a prolonged mother-young bond. This is illustrated by *Glossophaga soricina* a chiropteran with a complex feeding strategy, that has a low-energy, low-fat milk and slow development of the young (Jenness and Studier, 1976). The ringtail possums feeding behaviour involves food selection at four different levels (species, tree, leaf and leaf part) and is thought to enable it to maximise its intake of high quality food (Pahl, 1987b). Hence, the lactational strategy of the ringtail possum may also reflect the evolution of a browsing dietary habit where a prolonged relationship between mother and young may be advantageous to enable development of the young's feeding behaviour. In addition, development of other behaviours associated with the ringtail possums arboreal lifestyle (i.e., agility in the canopy) may require a long association between mother and young.

Bjornhag *et al.* (1979) suggests that there is a selective advantage to nurture young as efficiently as possible. In other words, the food resources available to the

mother are used for the growth of the young as efficiently as possible. The lactational strategy of the ringtail may not enable maximal growth of the young but represents the optimal condition for both mother and young. It has been selected to reduce the energy demands on the female and to prolong the mother-young bond, both of which may be necessary for life in the trees on a diet of leaves.

9.3 Comparison with other Mammalian Folivores

The rate of energy transfer from mother to suckling in other mammalian species may also reflect constraints imposed by diet and lifestyle. Within the family Carnivora, folivorous/herbivorous species (i.e., giant panda, *Ailuropoda melanoleuca*) have lower reproductive efforts relative to those of species in other trophic groups (Gittleman and Oftedal, 1987). The characteristics of ringtail possum milk (i.e., dilute with a low fat content) are shared by another marsupial folivore *T. vulpecula* (Cowan, 1989). Furthermore, the relatively long durations of lactation, patterns of parental care, slow rate of growth (Russell, 1982) and low 'offspring production rate' (Smith and Lee, 1984) of the marsupial folivores *Petauroides volans*, *Phascolarctos cinereus*, *Trichosurus vulpecula* strongly suggest a similar low energy investment in reproduction.

Compared with these other folivorous marsupial species the ringtail possum shows a relatively high annual fecundity and hence high maternal investment (Smith and Lee, 1984). The mass of the litter at weaning (expressed as a percentage of maternal body mass) is highest and the duration of lactation is shortest in the ringtail possum (Russell, 1982; Chapter 3). The combination of anatomical and physiological specialisations of the ringtail possums' digestive tract (Chilcott and Hume 1984a, b and c) and its behavioural feeding strategy (Pahl, 1987b; Chapter 1) may allow the ringtail possum to have a greater rate of intake of high quality food. This in turn would enable the rate of milk production by ringtail possum to be higher than that of other marsupial folivorous species. The koala, *Phascolarctos cinereus* has a particularly long duration of lactation and slow growth rate (Russell, 1982). This may reflect a relatively inefficient conversion of milk by the young koala dictated by its low basal metabolic rate (Degabriele and Dawson, 1979).

The reproductive traits of an average female ringtail possum breeding once a year, having a litter of two young and expending a relatively small proportion of her

total annual energy budget on reproduction should be matched with prolonged survival. Maximum longevity of the ringtail possum is 6 years (Thompson and Owen, 1964; Pahl, 1987a). This is in contrast to the relatively short life (1-3 years) but higher fecundity of dasyurid marsupials (Smith and Lee, 1984; Lee and Cockburn, 1985). However, other folivorous marsupials (i.e., *Trichosurus vulpecula*, *Petauroides volans*, *Phascolarctos cinereus*) live longer than the ringtail possum. This further suggests that the cost of reproduction to these species is less than that of the ringtail possum. However, other non-reproductive factors must also influence the longevity of a species (Smith and Lee, 1984). Studies of the reproductive energetics and lactational strategies of *Trichosurus vulpecula*, *Petauroides volans* and *Phascolarctos cinereus* are needed to clarify the reasons for the higher fecundity of the ringtail possum.

9.4 Reproductive Energetics and Variations in Reproductive Traits

The timing of births and annual fecundity varies both between and within ringtail possum populations (Thompson and Owen, 1964; Marsh, 1968; Hird, 1975; How *et al.*, 1984; Pahl and Lee, 1988; Chapter 3). The age structure and body mass of the females in the population are two factors responsible for variation in these parameters (Pahl and Lee, 1988). A limit to energy expenditure may be imposed by body size which would explain why smaller primiparous females usually give birth later, and tend to produce smaller litters, than heavier multiparous females (Thompson and Owen, 1964; Hird, 1975, How *et al.*, 1984, Pahl and Lee, 1988; Chapter 3). For example, young females may need to reach a critical body size before they can channel energy into fat reserves, and cope with the increased food energy intake, required for late lactation. Similarly, the ability of a multiparous female to acquire the additional energy for milk production may place a constraint on litter size since the amount of energy required for lactation can increase with increasing litter size (McClure, 1987; Kenagy, 1987).

Not all variation in the timing of births and annual fecundity is explained by body size. Pahl and Lee (1988) note that environmental factors (i.e., weather and food conditions) may also influence these reproductive traits. The present study suggests that the quality of the maternal diet may influence the concentration of

certain milk components (Chapters, 7,8). Differences between the chemical composition of the diet of ringtail possums in the wild compared with that of animals in captivity correlated with differences in milk composition. In particular, the concentration of fat was highest in milk collected from wild animals that were feeding predominantly on *Leptospermum laevigatum* which is higher in fibre and crude lipid than the diet of captive animals (Chapter, 4,5). Therefore, a female may produce a milk with a higher nutrient content by selecting particular leaves, subject to their availability. This in turn may enable a faster rate of growth by the young, leading to earlier weaning and the chance for the female to breed again within a year. Although no significant difference was found between the growth rate of wild and captive animals in this study this was probably due to the additional free-living energy requirements of the wild young (Chapter 3,8).

Alternatively selection of leaves with a high nutrient and available energy content may enable a higher rate of milk production by a particular female. Therefore, differences in the quality of leaves available between years, between areas of forest and within areas of forest may account for some of the variation in the reproductive traits of the ringtail possum. For example, the lower annual fecundity of the population of ringtail possums in coastal tea-tree scrub at Whitemark Beach (Chapter 3) compared with that of a population in Victoria (Pahl and Lee, 1988), inhabiting a younger area of scrub, may be related to differences in leaf quality between the two areas. However, studies of both reproductive energetics (including lactation) and the quality and availability of food in a range of ringtail possum populations are required to test this hypothesis.

9.5 Population Requirements

It is possible to make a preliminary estimate of the amount of food eaten by a population of ringtail possums in a *Leptospermum laevigatum* thicket from the data collected in this study. Using the values of feeding rates estimated in Chapter 6 (i.e., females = 46.87 kgleaves.kganimal⁻¹.yr⁻¹, males = 47.81 kgleaves.kganimal⁻¹.yr⁻¹) and the estimated food required to provide the potential energy in products that are synthesised by females (i.e., fat = 0.18 kgleaves.kganimal⁻¹.yr⁻¹, milk = 0.47 kgleaves.kganimal⁻¹.yr⁻¹, Table 9.1, Chapter 4), it can be calculated that an adult pair of ringtail possums (rearing a litter of two) would consume approximately

95.33 kgleaves.kganimal⁻¹.yr⁻¹. The biomass of adult ringtail possums at Whitemark Beach was estimated to be 2.1kg.ha⁻¹ from mean population density estimates and the mean body mass of adult ringtail possums (992.7g, Chapter 3). Therefore, the adult ringtail possums at Whitemark Beach consumed approximately 200.2 kgleaves (ha.yr)⁻¹.

The leaf production rate of *Leptospermum laevigatum* in Victoria is approximately, 5722.5 leaves.tree⁻¹.year⁻¹ (Pahl, 1987) and the measured mass of one *Leptospermum laevigatum* leaf is approximately 0.055g ± 0.01 (n = 10). Therefore, leaf production was estimated to be 52.5 leaves.branch⁻¹.yr⁻¹ or 3.0 g.branch⁻¹.yr⁻¹. Using this value and assuming the number of peripheral branches on *Leptospermum laevigatum* to be 250 (personal observation) the leaf production rate at Whitemark Beach was estimated to be 553 kgleaves (ha.yr)⁻¹. Hence, adult ringtail possums consume about 36% of the estimated leaf biomass produced each year. This appears to support the suggestion by Marples (1973) that food availability is unlikely to be a limiting factor for possum populations. However, although the food of the ringtail possum is seemingly abundant not all the leaves are potential food (Pahl, 1987b). Some may be out of reach, some may be indigestible and some may be inedible because they contain high levels of allelochemicals or insufficient amounts of nutrients (Cork and Pahl, 1984). The productivity of an area of forest in terms of the dietary quality of leaves available needs to be determined before an accurate estimate of the area required to sustain a population of ringtail possums can be made.

The requirements of a population throughout the year, not just during one particular season, are needed to effectively manage a species. This work on the ringtail possum shows that the most energetically demanding time for the female is during the late spring and early summer months. This time is also a critical stage for the sucklings whilst the nutritive value of the milk is at its lowest and the young is not yet adapted to a leaf diet. Therefore this is the time of year when the species would be most vulnerable to disturbance

9.6 Final Conclusions

Seasonal fluctuations in environmental conditions (i.e., climate and food quality) may result in variations in the energy and nutrients available to an animal

and may therefore effect an animals' daily energy expenditure and its ability to meet additional energy requirements. The ringtail possum appears to minimise the effect of changes in environmental factors on its daily energy budget by adopting energy conserving behaviours, such as nest sharing. In addition, the ringtail possum avoids potential large peaks in energy requirements associated with reproduction by spreading the energy demands of lactation over time and timing the most costly period to months when environmental energy demands are low and food quality may be high. The accumulation of energy reserves prior to the period of maximum energy demand also helps to reduce the effect of lactation on the daily energy budget of the female. The observation that a females breeding frequency may increase if conditions are favourable (e.g., increased food quality) suggests that intake rather than expenditure limits energy allocation to reproduction in the ringtail possum.

Previous studies show that the ringtail possum has evolved behavioural (Pahl, 1987b) and digestive strategies (Chilcott and Hume, 1984a,b,c) which maximise nutrient and energy intake from its poor quality diet. The present study has shown that the ringtail possum also exhibits behavioural, physiological and ecological strategies which enable it to minimise energy requirements whenever possible. Such adaptations appear to have evolved in response to a limit to the rate at which the ringtail possum can acquire energy from its leaf diet.

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APPENDIX A

Acari (Ticks) Collected From Ringtail Possums On Flinders Island

The following ticks were collected from the ears and facial region of adult ringtail possums caught in the study area on Flinders Island. The tick species and were identified by Dr. D.H. Kemp, C.S.I.R.O, Division of Tropical animal Science, Queensland.

Tick Species

Ixodes tasmani
Ixodes tasmani
Ixodes tasmani
Ixodes tasmani
Ixodes (Sternalixodes) sp.

possibly *Ixodes (S.) cornuatus*
Ixodes tasmani

APPENDIX B

Vocalisations of the Common Ringtail Possum

The following vocal sounds and the context in which they were emitted were recorded during the capture, mark and release (Chapter 2), and radio-surveillance (Chapter 4), of free-living ringtail possums at Whitemark Beach, Flinders Island. Sounds emitted by animals maintained in captivity were also noted.

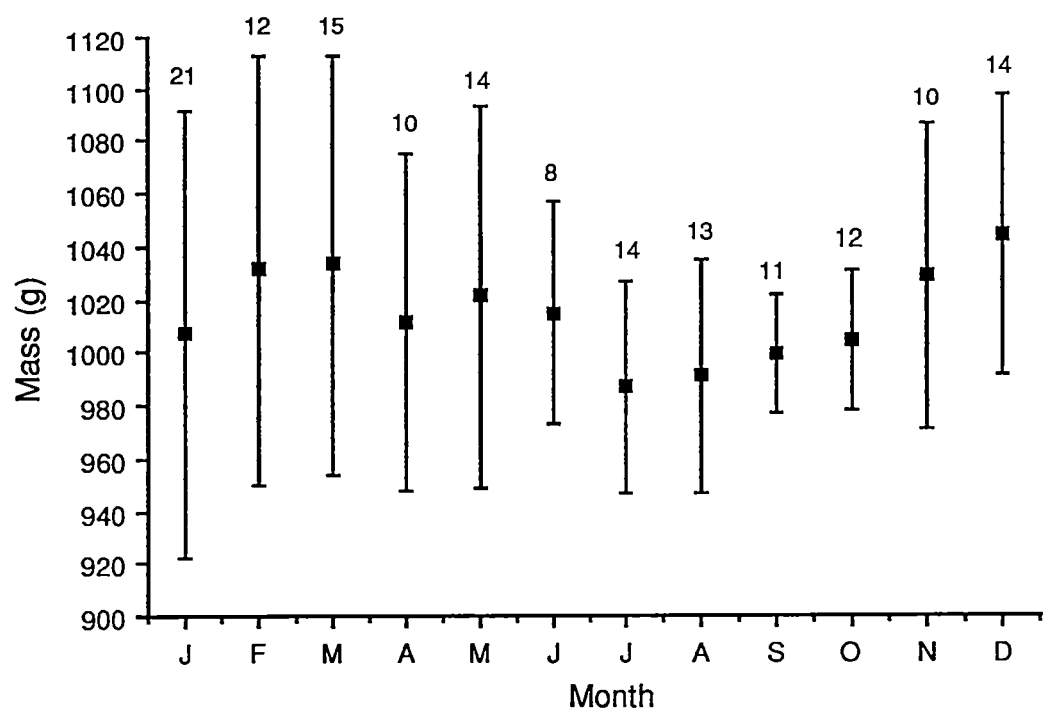
Description of Vocalisation	Context
Sonic twitter	This sound was produced by animals when disturbed, either by some natural disturbance while an animal was feeding, or during capture.
Chirruping	Emitted on numerous occasions, usually while animals fed. However, it was also produced by mothers whose back-young were feeding nearby. Chirruping calls were also exchanged between familiar conspecifics.
Rapid clicking	This sound was produced by adults of both sex when approaching an occupied nest box. The nest box occupant responded by partially emerging from the box sniffing the intruder and then either attacking the approaching animal or allowing it to enter the nest box. A loud clicking noise was emitted by a male when the female occupying the cage with him was in oestrus. This noise was accompanied by 'courtship' behaviour in which the male pursued the female around the cage.
"Chi-Chi-Chi"	This vocalisation was produced by sucklings when separated from their mother.

APPENDIX C

Body Composition of Pouch Young.

Three healthy pouch young, two aged 95 days (K and J, see Chapter 2) and one 65 days old (N, see Chapter 2) died during the measurement period (i.e., two were killed after their mother died under anaesthetic and the third died accidentally while in the incubator). Another two young (Q and R) died at 61 days of age when their primiparous mother ceased lactating. The carcasses of the young (K, J, N and Q) were used for dry matter determination by dessication at 60°C. The dried carcasses were then ground to a fine powder and subsamples used for estimation of body composition by K. Newgrain, Division of Wildlife and Ecology, C.S.I.R.O, Canberra. Total nitrogen was estimated by Kjeldahl digestion and Conway microdiffusion analysis, and total fat by Soxhlet extraction. The protein content of the carcass was determined by multiplying the total N values by 6.25.

Young	Age (days)	Mass (g)	Dry matter (% W/W)	Water (% W/W)	Fat (% W/W)	Protein (% W/W)
Q	61	4.4	16.84	83.10	0.26	10.28
N	65	12.9	17.59	82.40	3.05	9.58
K	95	38.3	20.78	79.22	4.74	11.14
J	95	36.4	24.17	75.83	5.40	13.92



APPENDIX D Seasonal cycle of body mass (g) of adult male ringtail possums maintained in captivity. Means \pm SD are given. N is above error bars.

APPENDIX E

Metabolic Rate and Water Influx of Ringtail Possums in Captivity.

Animal	Sex	Mean Mass (g)	Mass Change (%)	Metabolic Rate (ml CO ₂ /g/hr)	% Body Water	Water Influx (ml/kg/hr)
7	F*	860	+1.1	1.016	59.9	150.6
1	F	967	+0.7	1.196	71.8	132.1
4	F*	915	+0.2	1.288	66.6	121.6
2	F	1072	+0.1	1.129	65.8	117.4
1	F**	1035	+0.1		71.8	113.6
2	F**	1133	0.0		65.8	106.3
3	F**	1045	- 0.1		70.5	81.8
4	F	915	- 0.2		71.3	108.4
5	F	997	+0.5		76.4	86.7
6	M	1032	+0.6		71.0	108.3
Mean		997	+0.3	1.157	69.1	112.7
SD		± 83	± 0.4	± 0.114	± 4.6	± 20.1

* = Female suckling pouch young (Phase 2a of lactation).

** = Female suckling pouch young (Phase 2b of lactation).

Amendment

Since this thesis was completed the Tasmanian subspecies of the common ringtail possum has been changed from *Pseudocheirus peregrinus viverrinus* to *Pseudocheirus peregrinus convolutor* (Schinz, 1821).

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Nicholas K., Loughnan M., Messer M., Munks S., Griffiths M., Shaw D. (1989). Isolation, partial sequence and asynchronous appearance during lactation of lysozyme and alpha-lactalbumin in the milk of a marsupial, the common ringtail possum (*Pseudocheirus peregrinus*). *Comparative Biochemistry and Physiology. Part B: Biochemistry & Molecular Biology* 94(4):775-8